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Amyloid-$\beta_{1\text{-}42}$ Disrupts Synaptic Plasticity by Altering Glutamate Recycling at the Synapse

Edina Varga[a], Gábor Juhász[a], Zsolt Bozsó[a], Botond Penke[a], Lívia Fülöp[a] and Viktor Szegedi[b,*]
[a]*Department of Medical Chemistry, University of Szeged, Szeged, Hungary*
[b]*Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary*

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Abstract. Alzheimer's disease (AD) is the most prevalent form of neurodegenerative disorders characterized by neuritic plaques containing amyloid-β peptide (Aβ) and neurofibrillary tangles. Evidence has been reported that $A\beta_{1\text{-}42}$ plays an essential pathogenic role in decreased spine density, impairment of synaptic plasticity, and neuronal loss with disruption of memory-related synapse function, all associated with AD. Experimentally, $A\beta_{1\text{-}42}$ oligomers perturb hippocampal long-term potentiation (LTP), an electrophysiological correlate of learning and memory. Aβ was also reported to perturb synaptic glutamate (Glu)-recycling by inhibiting excitatory-amino-acid-transporters. Elevated level of extracellular Glu leads to activation of perisynaptic receptors, including NR2B subunit containing NMDARs. These receptors were shown to induce impaired LTP and enhanced long-term depression and proapoptotic pathways, all central features of AD. In the present study, we investigated the role of Glu-recycling on $A\beta_{1\text{-}42}$-induced LTP deficit in the CA1. We found that Aβ-induced LTP damage, which was mimicked by the Glu-reuptake inhibitor TBOA, could be rescued by blocking the NR2B subunit of NMDA receptors. Furthermore, decreasing the level of extracellular Glu using a Glu scavenger also restores TBOA or Aβ induces LTP damage. Overall, these results suggest that reducing ambient Glu in the brain can be protective against Aβ-induced synaptic disruption.

Keywords: Alzheimer's disease, glutamate scavenger, glutamate-reuptake, long-term potentiation, NR2B, TBOA

INTRODUCTION

Amyloid-β (Aβ), a misfolded peptide, is widely regarded as a central player in the pathogenesis of Alzheimer's disease (AD). The accumulation of soluble Aβ [1] in the brain of patients and animal models of AD is associated with impairments of cognition and memory [2–4]. In addition, both the synthetic and brain-derived soluble Aβ have been shown to damage certain forms of synaptic plasticity, correlates of learning and memory [5, 6]. Despite intense research, the mechanisms involved in Aβ-mediated neuronal degeneration and dysfunction are not well understood.

*Correspondence to: Viktor Szegedi, PhD., Biological Research Center – Biochemistry, Temesvari krt. 32, Szeged H-6726, Hungary. Tel.: +36 70 2418260; E-mail: szegedi.viktor@brc.mta.hu.

The hippocampus is especially affected in AD including hippocampal-dependent cognitive abilities such as learning and memory. Long-term potentiation (LTP), a form of synaptic plasticity in the CA1 field of the hippocampus, is impaired in animal models of AD. Numerous studies reported that $A\beta_{1\text{-}42}$ oligomers block hippocampal LTP *ex vivo* [7–9] and *in vivo* [10, 11].

Although the increased neuronal excitability caused by Aβ seems to contribute to and to be a key part of the pathomechanism of AD, the exact mechanisms by which neuronal over-activity develops is unknown. Glutamate (Glu) excitotoxicity has been established to have a major role in AD pathogenesis; however, how Aβ induces its effects is poorly understood. Numerous findings confirmed that excitotoxic effects of Glu contribute to progressive neuronal loss in AD [12–14]. Inhibited excitatory-amino-acid-transporters

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(EAATs) may be a central player in this mechanism, and indeed, recent findings suggest that Aβ oligomers perturb synaptic plasticity by altering Glu-recycling at the synapse [15, 16], resulting in elevated ambient extracellular Glu-level in the brain [17, 18], which might be responsible for the overexcitation seen in AD. Aβ blocks Glu-reuptake by inhibiting both neuronal and glial Glu transporters [16, 19], which might lead to extrasynaptic NMDAR (esyn NMDAR) activation. Esyn NMDAR activation causes inhibited LTP [5], enhanced long-term depression (LTD) [20], and apoptosis [21].

The aim of this study was to confirm that Aβ causes synaptic Glu-spillover and esyn NMDAR activation, which leads to impaired synaptic plasticity in the CA1. We show that blocking Glu-reuptake with TBOA also impairs LTP, and both TBOA- and Aβ-induced synaptic damage could be rescued by blocking NR2B subunit. Moreover, reducing the level of extracellular Glu by applying a glutamate-scavenger enzyme GPT also provides protection against impaired synaptic plasticity by TBOA and Aβ.

MATERIALS AND METHODS

Compounds

For the preparation of artificial cerebrospinal fluid (ACSF), all salts, glucose, sodium pyruvate (Pyr), glutamic-pyruvic transaminase (GPT), DL-threo-β-benzyloxyaspartate (TBOA), and α-(4-Hydroxyphenyl)-β-methyl-4- benzyl-1-piperidineethanol (+)-tartrate salt (ifenprodil) were purchased from Sigma-Aldrich (St. Louis, MO). $A\beta_{1\text{-}42}$ was synthetized at the Department of Medical Chemistry University of Szeged, Hungary. Detailed description of the synthesis and characterization of $A\beta_{1\text{-}42}$ is reported in [7].

Animals

The study conformed to EU Directive 2010/63/EU and was approved by the regional Station for Animal Health and Food Control under Project License XVI/8/2013. BALB/c mice were housed in groups of 2-3 under standard conditions (24°C, 12-h light-dark cycle) with food and water available *ad libitum*.

Ex vivo electrophysiology

Hippocampal slices of 400 μm in thickness were prepared from the brains of 3-month old mice using a standard protocol [22]. Briefly, slices were incubated in ACSF gassed with 95% O_2, 5% CO_2 at 35°C for 60 min. ACSF was composed of (in mM) 130 NaCl, 3.5 KCl, 3 $CaCl_2$, 1.5 $MgSO_4$, 0.96 NaH_2PO_4, 24 $NaHCO_3$, and 10 D-glucose, pH 7.4. Individual slices were transferred to a 3D-MEA chip with 60 tip-shaped and 60 μm high electrodes spaced by 200 μm (Qwane Biosciences, Lausanne, Switzerland). The surrounding solution was quickly removed, and the slice was immobilized by placing a grid onto it. The slice was continuously perfused with oxygenated ACSF (3 ml/min at 36°C) throughout the entire recording session. Unfiltered data were recorded using a standard, commercially available MEA 60 setup (Multi Channel Systems MCS GmbH, Reutlingen, Germany). Field excitatory postsynaptic potentials (fEPSPs) were recorded from the proximal stratum radiatum at 5 kHz.

Stimulation protocol

The Schaffer-collateral was stimulated by injecting a biphasic voltage waveform (–100/+100 μs) through one selected electrode at 0.033 Hz. Care was taken to place the stimulating electrode in the same region at every slice. The peak-to-peak amplitudes of fEPSPs at the proximal stratum radiatum of CA1 were analyzed. After a 30-min incubation period, the threshold and maximum stimulation intensities for evoked responses were determined. To evoke responses, 30% of the maximal stimulation intensity was used. LTP was evoked by theta-burst stimulation (TBS). TBS comprised of 15 bursts given at 5 Hz and individual burst contained 4 pulses given at 100 Hz per burst. The level of LTP was compared to the average of the last 10 peak-to-peak amplitudes of evoked fEPSPs before TBS.

Drug treatments

After 10-min control level, slices were treated with 1 μM $A\beta_{1\text{-}42}$ or 5 μM TBOA for 60-min before LTP was induced. Other cohort of slices was treated with 3 μM ifenprodil or 0.82 mM Pyr for 10-min then 2.06 U/ml GPT for 60-min before LTP induction. Separate groups of slices were treated with these compounds together with $A\beta_{1\text{-}42}$ or TBOA.

Statistics

Statistical significance was determined by using ANOVA on ranks test with the *post hoc* Dunn's method (SigmaPlot 11 software package). The p value ≤ 0.05 was considered significant in all cases.

RESULTS

A$\beta_{1\text{-}42}$-impaired LTP requires NR2B activation

We recorded fEPSPs from the stratum radiatum of the CA1 using MEA electrodes. The peak-to-peak amplitudes of fEPSPs were analyzed from the proximal part of stratum radiatum.

First, we verified the effect of A$\beta_{1\text{-}42}$ preparation on LTP in the hippocampal slices. Untreated slices showed a persistent elevated level of fEPSPs after LTP induction ($168.33 \pm 5.58\%$, $n = 12$), while A$\beta_{1\text{-}42}$ reduced the magnitude of LTP ($124.35 \pm 4.88\%$, $n = 9$, $p < 0.05$, nonparametric ANOVA, Dunn *post-hoc* test; Fig. 1). Several recent studies suggested that different NR2 subunits of NMDARs may have divergent roles in NMDAR-dependent LTP activation and Aβ pathology (see discussion). To test whether LTP activation requires NR2B-containing NMDARs function, slices were treated with an NR2B antagonist, ifenprodil. We observed that ifenprodil did not alter the level of LTP compared to control ($176.81 \pm 4.93\%$, $n = 5$), suggesting that NR2B-activation is not required for LTP in the CA1. Furthermore, ifenprodil prevents the A$\beta_{1\text{-}42}$ effect on LTP ($166.03 \pm 12.38\%$, $n = 5$, $p < 0.05$, ANOVA on ranks, Dunn *post-hoc* test; Fig. 1), suggesting A$\beta_{1\text{-}42}$ induce LTP damage is via NR2B-containing NMDARs. None of the applied compounds altered the amplitude of fEPSPs during the wash-in period.

Glu-scavenger rescues the A$\beta_{1\text{-}42}$-impaired LTP

To determine whether A$\beta_{1\text{-}42}$ affects Glu-reuptake, we used an enzymatic Glu-scavenger system to reduce extracellular Glu-levels. Slices were treated

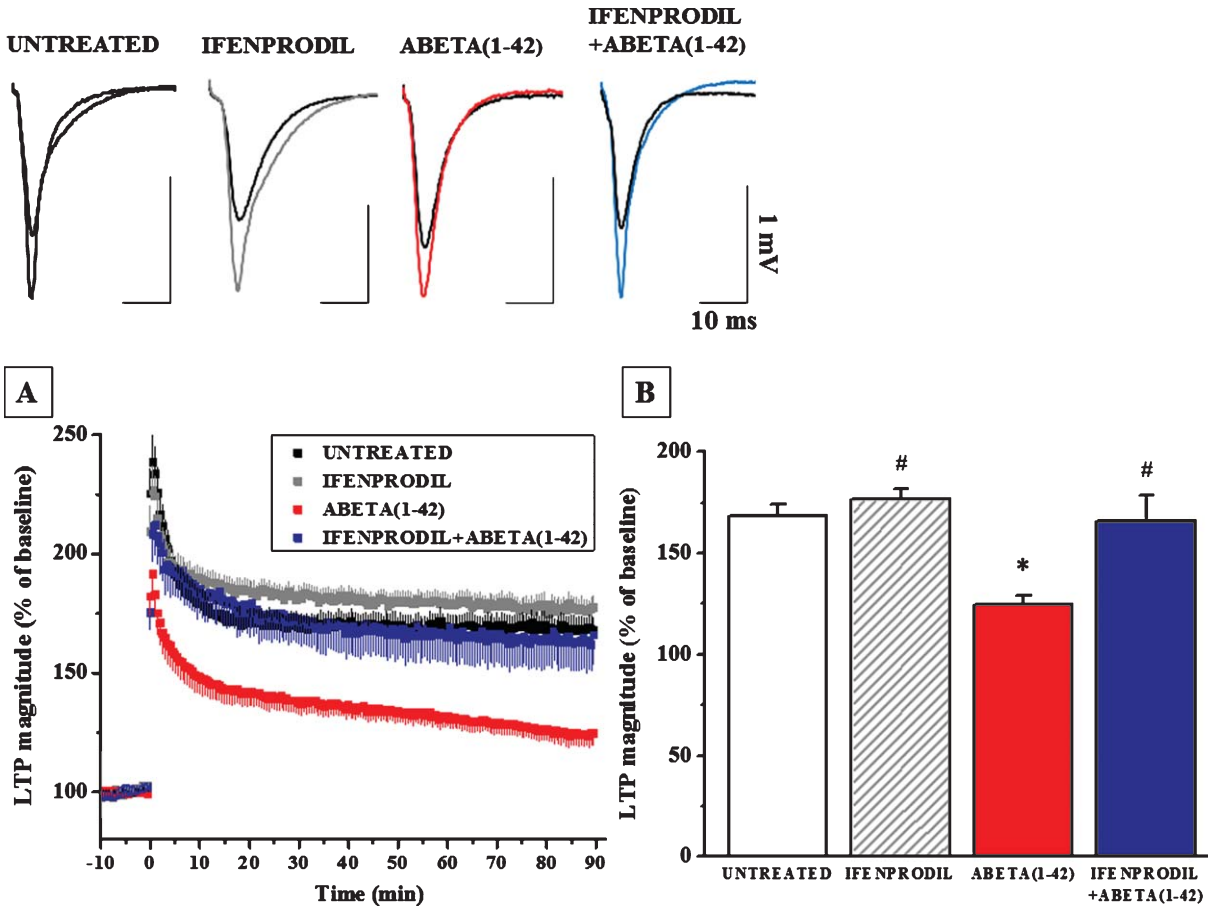

Fig. 1. Blocking NR2B subunit prevents A$\beta_{1\text{-}42}$-induced LTP damage. Insets show representative fEPSPs before (black) and after treatment. LTP was altered in A$\beta_{1\text{-}42}$ treated slices compared to untreated group (untreated versus A$\beta_{1\text{-}42}$: $^{*}p < 0.05$; ANOVA on ranks, Dunn *post-hoc* test). Ifenprodil did not change the level of LTP, however, it rescued the A$\beta_{1\text{-}42}$-impaired LTP. Error bars represent SEM; $^{\#}p < 0.05$ versus A$\beta_{1\text{-}42}$.

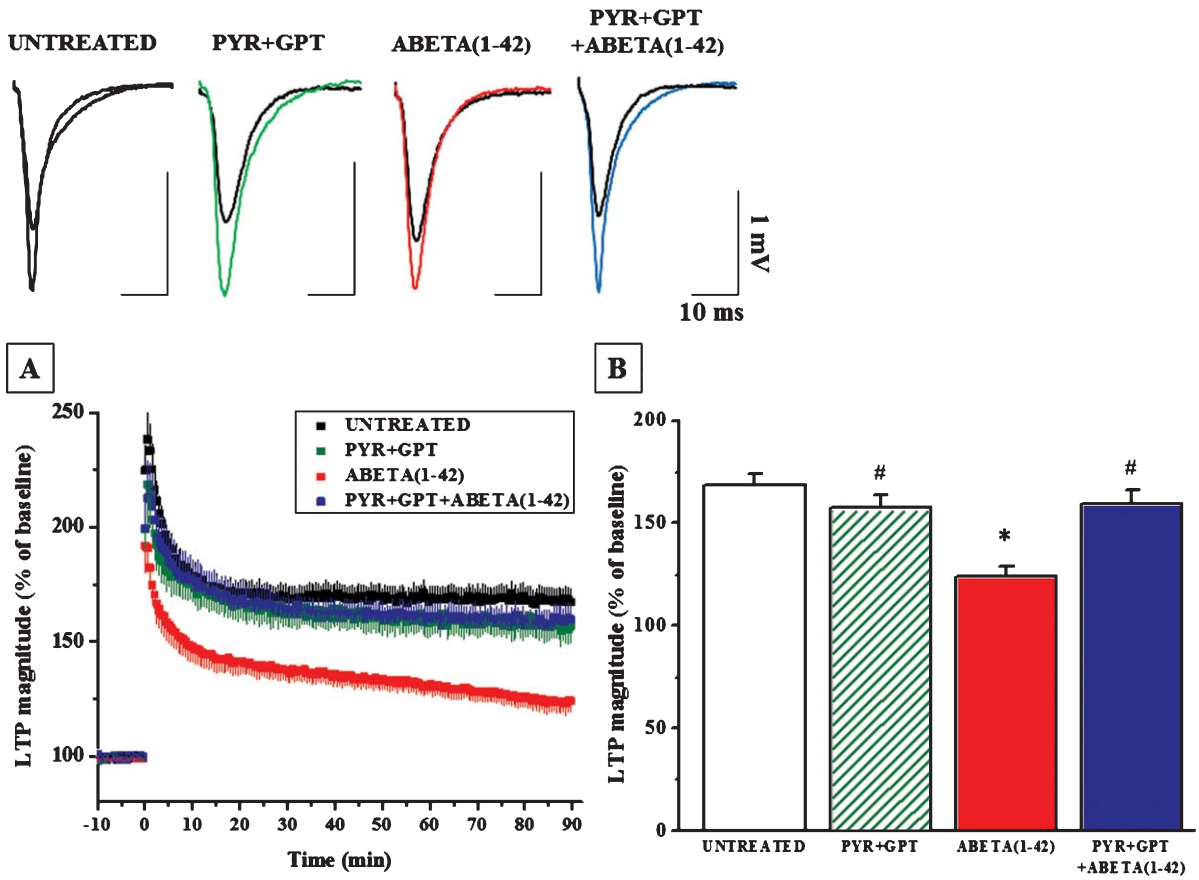

Fig. 2. Glu-scavenger restores $A\beta_{1\text{-}42}$-induced LTP damage. Insets show representative fEPSPs before (black) and after treatment. Pyr+GPT treatment did not affect the level of LTP compared to untreated slices, however $A\beta_{1\text{-}42}$ induced LTP impairment was prevented by Glu-scavenger ($A\beta_{1\text{-}42}$ versus Pyr+GPT+$A\beta_{1\text{-}42}$: $^{\#}p<0.05$; ANOVA on ranks, Dunn *post-hoc* test). Error bars represent SEM; $^{*}p<0.05$ versus untreated; $^{\#}p<0.05$ versus $A\beta_{1\text{-}42}$.

with GPT and its substrate, Pyr for 10 min followed by $A\beta_{1\text{-}42}$ for 60 min, then LTP was induced. We have found that Pyr+GPT treatment does not affect the level of LTP compared to control slices ($157.32 \pm 6.68\%$, $n=5$; Fig. 2). However, $A\beta_{1\text{-}42}$-induced LTP damage was prevented by Glu-scavenger (Pyr+GPT+$A\beta_{1\text{-}42}$: $159.66 \pm 6.37\%$, $n=5$ versus $A\beta_{1\text{-}42}$, $p<0.05$, ANOVA on ranks, Dunn *post-hoc* test; Fig. 2).

The effect of $A\beta_{1\text{-}42}$ is mimicked by TBOA, a Glu-reuptake inhibitor

TBOA was applied for 60 min before LTP induction. LTP was impaired by TBOA compared to untreated slice ($123.22 \pm 3.48\%$, $n=6$, $p<0.05$; ANOVA on ranks, Dunn *post-hoc* test, Fig. 3). Next, we tested whether NR2B subunit activation is required for the effect of TBOA. We have found that ifenprodil prevents TBOA-induced LTP damage suggesting NR2B subunit activation is essential for the effect of TBOA on LTP (ifenprodil+TBOA: $159.29 \pm 10.67\%$, $n=4$ versus TBOA: $p<0.05$; ANOVA on ranks, Dunn *post-hoc* test, Fig. 3). We proceeded to apply Glu-scavenger to test whether the inhibitory effect of TBOA was due to the elevated extracellular Glu-level. Indeed, TBOA-failed to impair LTP after Glu-scavenger treatment (Pyr+GPT+TBOA: 169.28 ± 8.18, $n=5$ versus TBOA, $p<0.05$; ANOVA on ranks, Dunn *post-hoc* test, Fig. 4). Collectively these results suggest that TBOA and Aβ share common pathway in synaptotoxicity. The effect of $A\beta_{1\text{-}42}$ is mimicked by Glu-reuptake inhibition; however both could be prevented by a Glu-scavenger and NR2B inhibition suggesting that $A\beta_{1\text{-}42}$ disrupts synaptic plasticity by altering Glu-recycling at the synapse in the CA1. Again, none of the applied compounds altered fEPSP amplitude during the wash-in period.

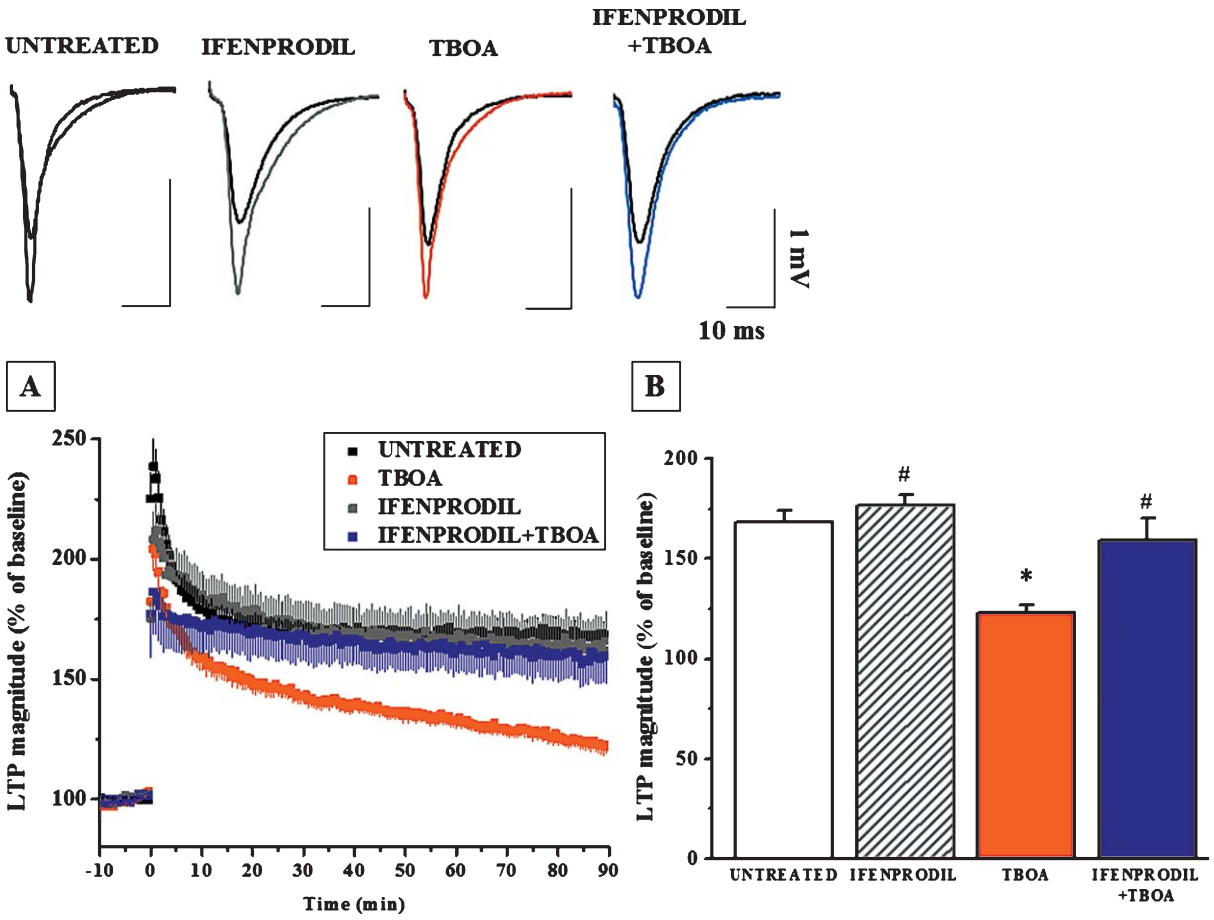

Fig. 3. Ifenprodil prevents TBOA-impaired LTP. Insets show representative fEPSPs before (black) and after treatment. LTP was impaired by TBOA compared to untreated group (untreated versus TBOA: $^{*}p < 0.05$; ANOVA on ranks, Dunn *post-hoc* test), however TBOA-induced LTP impairment was restored by ifenprodil (TBOA versus ifenprodil+TBOA: $^{\#}p < 0.05$; ANOVA on ranks, Dunn *post-hoc* test). Error bars represent SEM; $^{*}p < 0.05$ versus untreated; $^{\#}p < 0.05$ versus TBOA.

DISCUSSION

There is growing evidence that soluble Aβ oligomers mediate synaptic impairment in AD, but the exact mechanism of synaptotoxicity remains to be determined. Numerous studies have reported that Aβ can affect the function of NMDARs [23–27], which may lead to excitotoxicity and neuronal hyperactivation seen in the early stage of AD. Recent findings suggest that Aβ binds to prion protein, metabotropic Glu receptor 5, and integrin receptors, and this complex initiates a molecular cascade mediated by fyn kinase [28–30], which subsequently phosphorylates NMDARs.

An additional pathway of Aβ-mediated hyperexcitation could be, however, that the concentration of extracellular Glu is increased by Aβ. We have shown previously, that the excitatory effect of Aβ, as was determined by the rate of spontaneous spiking in hippocampal slices, is mediated by extrasynaptic NMDARs [22]. In the present study, we show that Aβ causes Glu spillover and subsequent esyn NMDAR activation, which could be prevented by either NR2B blockade or by "mopping-up" Glu with a Glu-scavenger enzyme.

TBOA mimics the effects of Aβ

Aβ has been shown to elevate extracellular Glu concentration in the brain without altering gamma-aminobutyric acid (GABA) level [18]. The mechanism behind this is probably the inhibition of the transporters mediating Glu-clearance. The level of brain extracellular Glu is regulated by EAATs expressed mainly on the astrocytes, which efficiently remove the excess of this neurotransmitter from the synaptic

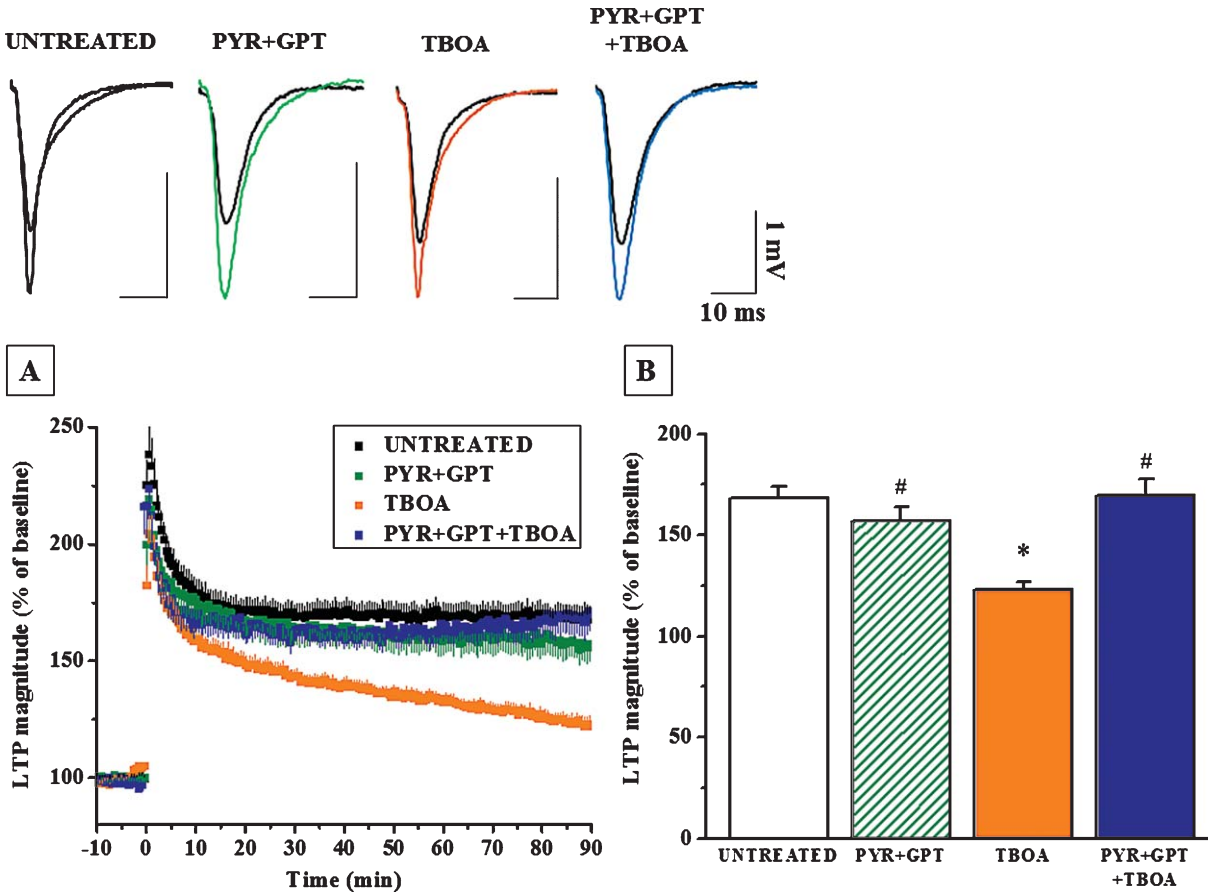

Fig. 4. Glu-scavenger prevents TBOA-induced LTP damage. Insets show representative fEPSPs before (black) and after treatment. TBOA-induced LTP impairment was restored by Glu-scavenger (TBOA versus Pyr+GPT+TBOA: $^{\#}p < 0.05$; ANOVA on ranks, Dunn *post-hoc* test). Error bars represent SEM; $^{*}p < 0.05$ versus untreated; $^{\#}p < 0.05$ versus TBOA.

cleft (reviewed by [31]). Indeed, Aβ was shown to inhibit both glial and neuronal EAATs [16, 17, 32]. Moreover, Noda and coworkers have reported that Aβ may even cause reverse functioning of EAAT, leading to Glu-release from glial cells [33]. Furthermore, down-regulation or abnormal expression and protein levels of EAAT1 and EAAT2 are altered in the hippocampus and frontal cortex of AD patients [34, 35] and in amyloid-β protein precursor transgenic mice [32], further supporting that Glu-level regulation fails during AD pathomechanism. These effects may lead to Glu-spillover from the synapses and subsequent activation of esyn receptors. Of particular interest here, esyn NMDARs containing mainly NR2B subunit were shown to activate apoptotic pathways and promote synaptic depression [21]. In contrast, NR2A subunit-containing NMDARs localized mainly at the synaptic domain are antiapoptotic and participate in the induction of LTP in the CA1. We previously reported that $A\beta_{1-42}$ induces hyperexcitability via NR2B-containing NMDARs [22]. Indeed, blocking selectively NR2B subunits protected against Aβ effects (including LTP impairment), suggesting that NR2B could be a promising target against AD [5, 8, 9, 36, 37]. Tackenberg et al. also suggest that esyn NR2B-containing NMDARs activation is essential for tau-dependent neurodegeneration [26]. It was also shown that the opposite form of synaptic plasticity, LTD requires both syn and esyn receptor activation [20], while LTP is not mediated by esyn NMDARs [8, 9]. It should be noted, however, that contradictory data is available on the role of NR2B NMDARs on LTP: esyn NDMARs are also recruited to the synapse during LTP [38] and may play an essential role in LTP maintenance [39, 40].

Our results extend this line of research by showing that reducing Glu that has been spilled-over from the synapse with a Glu-scavenger enzyme also prevents

Aβ induced LTP impairment. Moreover, we show that blocking EAATs by a selective inhibitor, TBOA, mimics the effects of Aβ: both compounds impair LTP, and this could be prevented by ifenprodil or GPT (Glu-scavenger).

Reducing ambient Glu in the brain is protective against Aβ induced LTD enhancement [16]. A recent paper by Chen and Herrup makes the suggestion that although the level of glutamine-synthase is elevated in AD brains, its activity is severely compromised by oxidative damage, leading to impaired Glu-metabolism [41].

Aβ and Glu-toxicity mediated dysfunction has been closely associated; however, decreasing the extracellular Glu-level can be protective in other conditions such as brain ischemia [42], stroke [43], traumatic brain injury [44] or certain psychiatric disorders [45, 46].

CONCLUSIONS

Collectively, our results provide evidence that Aβ impair Glu-recycling at the synapse, which leads to Glu-spillover and NR2B activation. Blocking NR2B or decreasing extracellular Glu offers protection against the synaptic plasticity impairment caused by Aβ.

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