

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

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**Abstract.** Alzheimer's disease (AD) is the most prevalent form of neurodegenerative disorders characterized by neuritic plaques containing amyloid- $\beta$  peptide (A $\beta$ ) and neurofibrillary tangles. Evidence has been reported that A $\beta_{1-42}$  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 $\beta_{1-42}$  oligomers perturb hippocampal long-term potentiation (LTP), an electrophysiological correlate of learning and memory. A $\beta$  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 $\beta_{1-42}$ -induced LTP deficit in the CA1. We found that A $\beta$ -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 A $\beta$  induces LTP damage. Overall, these results suggest that reducing ambient Glu in the brain can be protective against A $\beta$ -induced synaptic disruption.

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

## INTRODUCTION

Amyloid- $\beta$  (A $\beta$ ), a misfolded peptide, is widely regarded as a central player in the pathogenesis of Alzheimer's disease (AD). The accumulation of soluble A $\beta$  [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 A $\beta$  have been shown to damage certain forms of synaptic plasticity, correlates of learning and memory [5, 6]. Despite intense research, the mechanisms involved in A $\beta$ -mediated neuronal degeneration and dysfunction are not well understood.

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 $\beta_{1-42}$  oligomers block hippocampal LTP *ex vivo* [7–9] and *in vivo* [10, 11].

Although the increased neuronal excitability caused by A $\beta$  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 A $\beta$  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 A $\beta$  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. A $\beta$  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 A $\beta$  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 A $\beta$ -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 A $\beta$ .

## MATERIALS AND METHODS

### Compounds

For the preparation of artificial cerebrospinal fluid (ACSF), all salts, glucose, sodium pyruvate (Pyr), glutamic-pyruvic transaminase (GPT), DL-threo- $\beta$ -benzyloxyaspartate (TBOA), and  $\alpha$ -(4-Hydroxyphenyl)- $\beta$ -methyl-4-benzyl-1-piperidine-ethanol (+)-tartrate salt (ifenprodil) were purchased from Sigma-Aldrich (St. Louis, MO). A $\beta_{1-42}$  was synthesized at the Department of Medical Chemistry University of Szeged, Hungary. Detailed description of the synthesis and characterization of A $\beta_{1-42}$  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  $\mu$ m 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<sub>2</sub>, 5% CO<sub>2</sub> at 35°C for 60 min. ACSF was composed of (in mM) 130 NaCl, 3.5 KCl, 3 CaCl<sub>2</sub>, 1.5 MgSO<sub>4</sub>, 0.96 NaH<sub>2</sub>PO<sub>4</sub>, 24 NaHCO<sub>3</sub>, and 10 D-glucose, pH 7.4. Individual slices were transferred to a 3D-MEA chip with 60 tip-shaped and 60  $\mu$ m high electrodes spaced by 200  $\mu$ m (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  $\mu$ s) 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  $\mu$ M A $\beta_{1-42}$  or 5  $\mu$ M TBOA for 60-min before LTP was induced. Other cohort of slices was treated with 3  $\mu$ M 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 $\beta_{1-42}$  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  $\leq 0.05$  was considered significant in all cases.

## RESULTS

### $A\beta_{1-42}$ -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  $A\beta_{1-42}$  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  $A\beta_{1-42}$  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  $A\beta$  pathology (see discussion). To test whether LTP acti-

vation 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  $A\beta_{1-42}$  effect on LTP ( $166.03 \pm 12.38\%$ ,  $n = 5$ ,  $p < 0.05$ , ANOVA on ranks, Dunn *post-hoc* test; Fig. 1), suggesting  $A\beta_{1-42}$  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 $A\beta_{1-42}$ -impaired LTP

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

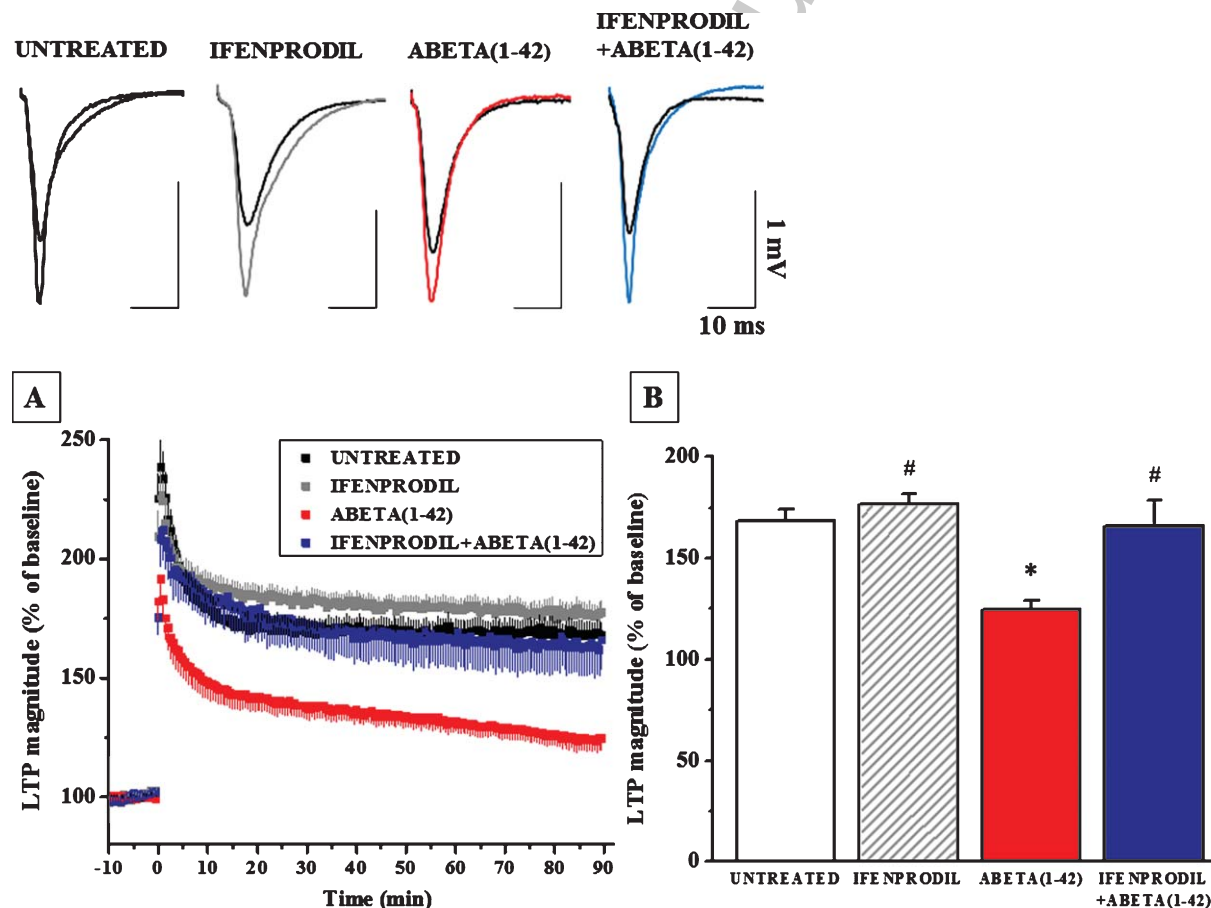


Fig. 1. Blocking NR2B subunit prevents  $A\beta_{1-42}$ -induced LTP damage. Insets show representative fEPSPs before (black) and after treatment. LTP was altered in  $A\beta_{1-42}$  treated slices compared to untreated group (untreated versus  $A\beta_{1-42}$ :  $*p < 0.05$ ; ANOVA on ranks, Dunn *post-hoc*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

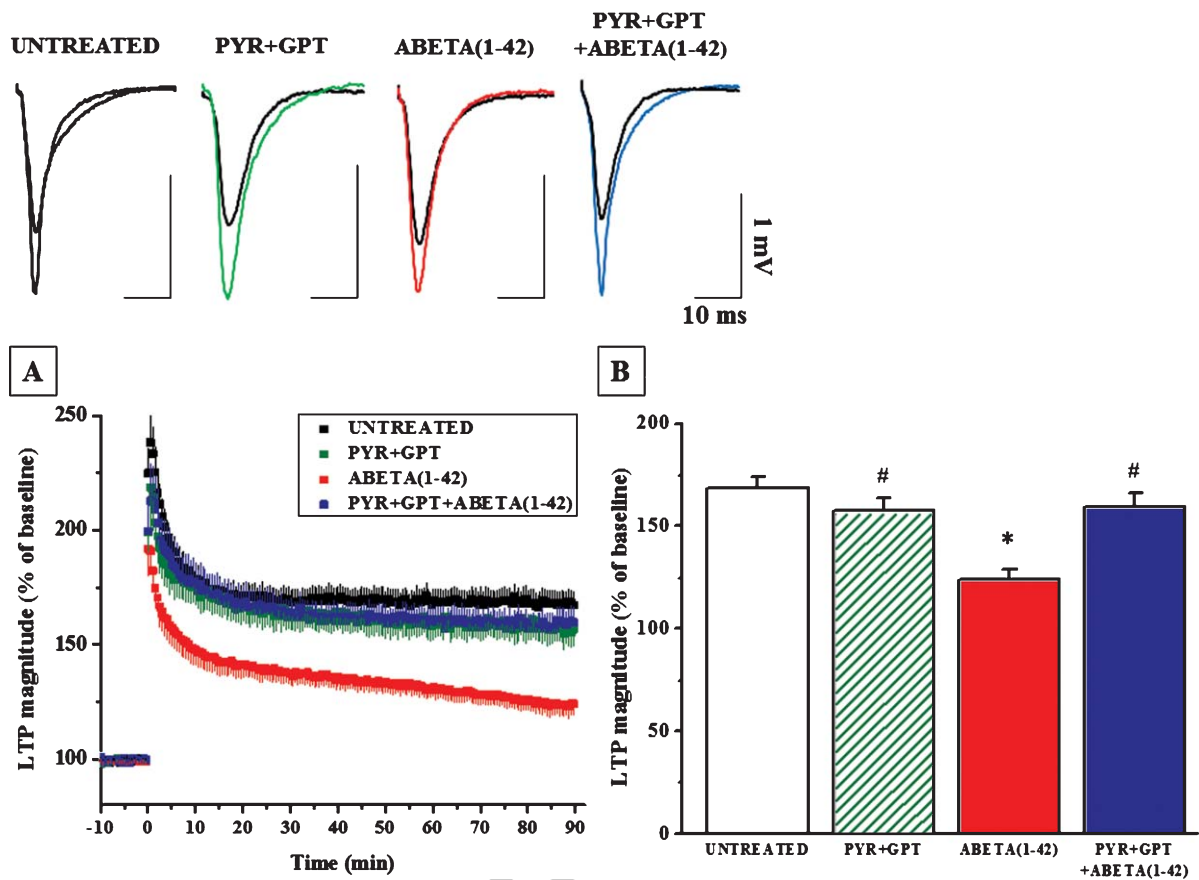


Fig. 2. Glu-scavenger restores  $A\beta_{1-42}$ -induced LTP damage. Insets show representative fEPSPs before (black) and after treatment. Pyr+GPT treatment did not affect the level of LTP compared to untreated slices, however  $A\beta_{1-42}$  induced LTP impairment was prevented by Glu-scavenger (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

with GPT and its substrate, Pyr for 10 min followed by  $A\beta_{1-42}$  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,  $A\beta_{1-42}$ -induced LTP damage was prevented by Glu-scavenger (Pyr+GPT+ $A\beta_{1-42}$ :  $159.66 \pm 6.37\%$ ,  $n = 5$  versus  $A\beta_{1-42}$ ,  $p < 0.05$ , ANOVA on ranks, Dunn *post-hoc* test; Fig. 2).

#### The effect of $A\beta_{1-42}$ 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:  $169.28 \pm 8.18\%$ ,  $n = 5$  versus TBOA,  $p < 0.05$ ; ANOVA on ranks, Dunn *post-hoc* test, Fig. 4). Collectively these results suggest that TBOA and  $A\beta$  share common pathway in synaptotoxicity. The effect of  $A\beta_{1-42}$  is mimicked by Glu-reuptake inhibition; however both could be prevented by a Glu-scavenger and NR2B inhibition suggesting that  $A\beta_{1-42}$  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.

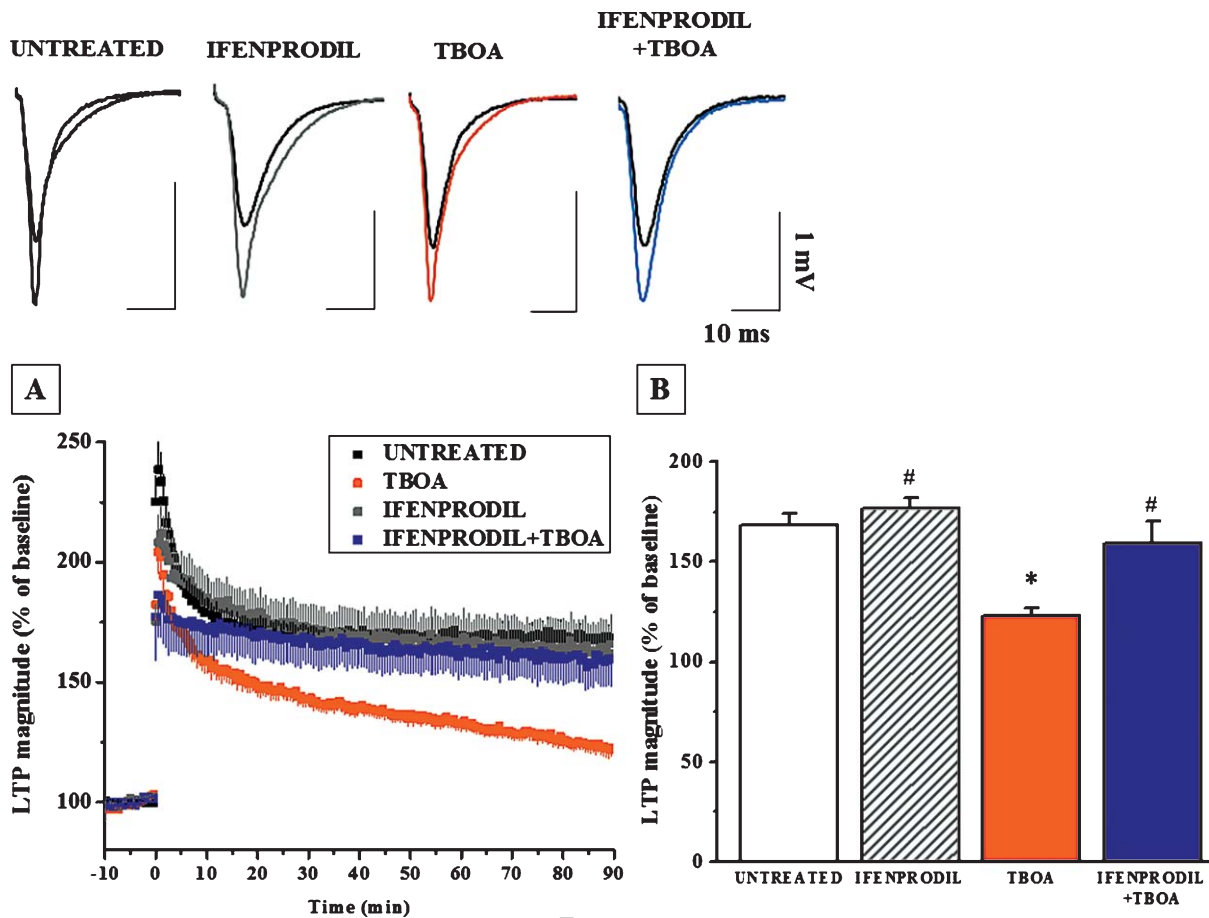


Fig. 3. Ifenprodil prevents TBOA-impaired LTP. Insets show representative fEPSPs before (black) and after treatment. LTP was impaired by TBOA compared to untreated group (untreated versus TBOA:  $*p < 0.05$ ; ANOVA on ranks, Dunn *post-hoc* test), however TBOA-induced LTP impairment was restored by ifenprodil (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 A $\beta$  oligomers mediate synaptic impairment in AD, but the exact mechanism of synaptotoxicity remains to be determined. Numerous studies have reported that A $\beta$  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 A $\beta$  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 A $\beta$ -mediated hyperexcitation could be, however, that the concentration of extracellular Glu is increased by A $\beta$ . We have shown previously, that the excitatory effect of A $\beta$ , 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 A $\beta$  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 A $\beta$

A $\beta$  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

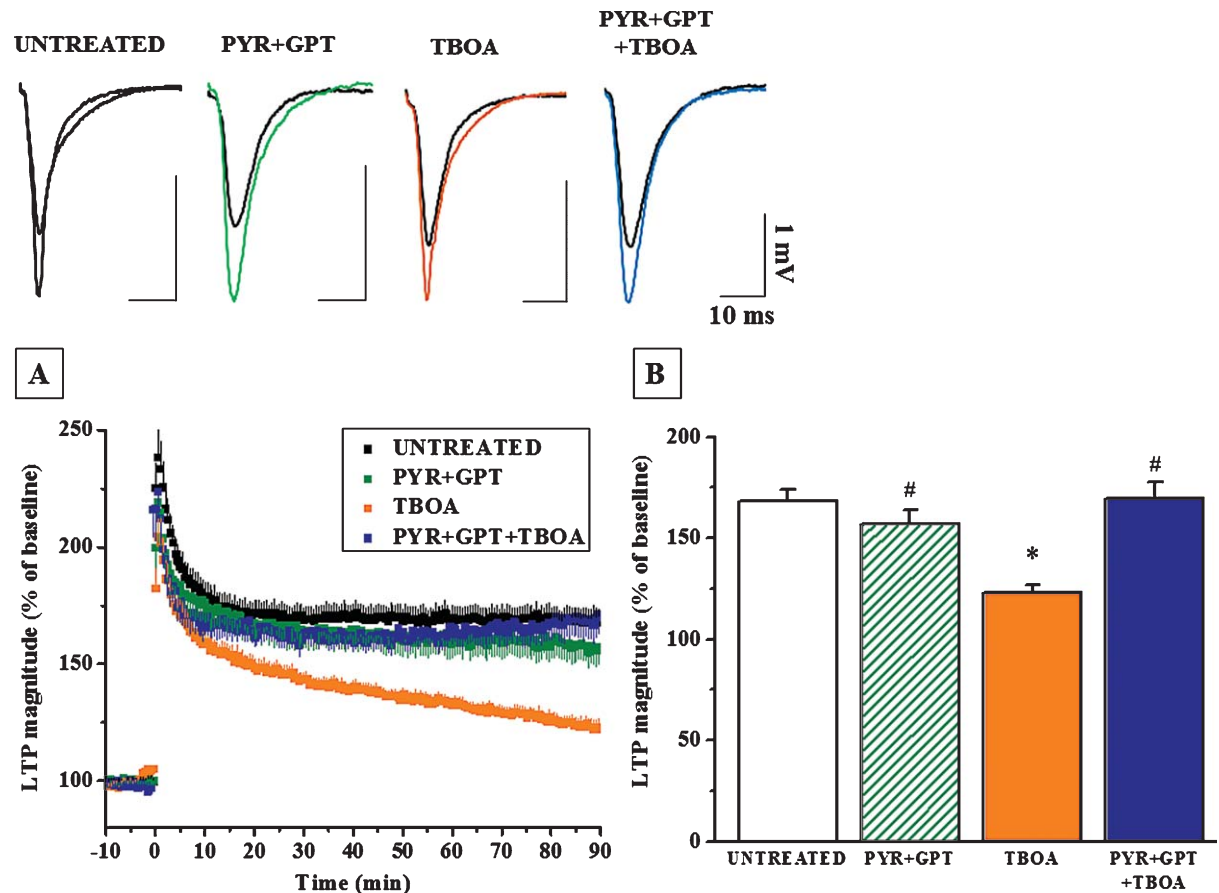


Fig. 4. Glu-scavenger prevents TBOA-induced LTP damage. Insets show representative fEPSPs before (black) and after treatment. TBOA-induced LTP impairment was restored by Glu-scavenger (TBOA versus Pyr+GPT+TBOA:  $^{\#}p < 0.05$ ; ANOVA on ranks, Dunn *post-hoc* test). Error bars represent SEM;  $^*p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

cleft (reviewed by [31]). Indeed, A $\beta$  was shown to inhibit both glial and neuronal EAATs [16, 17, 32]. Moreover, Noda and coworkers have reported that A $\beta$  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- $\beta$  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 previ-

ously reported that A $\beta_{1-42}$  induces hyperexcitability via NR2B-containing NMDARs [22]. Indeed, blocking selectively NR2B subunits protected against A $\beta$  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 NMDARs 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



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

Reducing ambient Glu in the brain is protective against A $\beta$  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].

A $\beta$  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 A $\beta$  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 A $\beta$ .

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