

The role of STEP in Alzheimer disease

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Amyloid beta (A β), the putative causative agent in Alzheimer disease, is known to affect glutamate receptor trafficking. Previous studies have shown that A β downregulates the surface expression of N-methyl D-aspartate type glutamate receptors (NMDARs) by the activation of STriatal-Enriched protein tyrosine Phosphatase 61 (STEP₆₁). More recent findings confirm that STEP₆₁ plays an important role in A β -induced NMDAR endocytosis. STEP levels are elevated in human AD prefrontal cortex and in the cortex of several AD mouse models. The increase in STEP₆₁ levels and activity contribute to the removal of GluN1/GluN2B receptor complexes from the neuronal surface membranes. The elevation of STEP₆₁ is due to disruption in the normal degradation of STEP₆₁ by the ubiquitin proteasome system. Here, we briefly discuss additional studies in support of our hypothesis that STEP₆₁ contributes to aspects of the pathophysiology in Alzheimer's disease. Exogenous application of A β -enriched conditioned medium (7PA2-CM) to wild-type cortical cultures results in a loss of GluN1/GluN2B subunits from neuronal membranes. Abeta-mediated NMDAR internalization does not occur in STEP knock-out cultures, but is rescued by the addition of active TAT-STEP to the cultures prior to A β treatment.

In Alzheimer disease (AD), the abnormal accumulation of soluble A β peptides has a profound impact on cognitive function.¹

A β peptides disrupt synaptic plasticity, a molecular mechanism involved in learning and memory.^{2,3} N-methyl D-aspartate type glutamate receptors (NMDAR) play an important role in the development of synaptic strengthening. A β downregulates the surface expression of NMDARs by activation of STriatal-Enriched protein tyrosine Phosphatase 61 (STEP₆₁).⁴ STEP₆₁ is a brain-specific phosphatase that opposes the development of synaptic strengthening.⁵ STEP₆₁ is present in post-synaptic terminals, immunoprecipitates with the NMDAR complex and decreases NMDA channel function.^{6,7} The reduced channel function is mediated, at least in part, by an increased internalization of the NMDAR complex, as STEP dephosphorylates the GluN2B subunit at a regulatory tyrosine (tyr¹⁴⁷²) leading to NMDAR endocytosis. Knocking down STEP with interfering RNA increases NMDAR trafficking to synaptic membranes.^{4,8} A previous study suggested that A β leads to the activation of STEP through a calcineurin-mediated pathway, which subsequently increased internalization of surface NMDAR.⁴ A recent study has demonstrated that STEP is also regulated by the ubiquitin proteasome system, and an A β -mediated disruption of the proteasome leads to increased STEP₆₁ levels in human Alzheimer's disease (AD) brains and AD mouse models.⁹ Taken together, these studies suggest that an increase in the activity of STEP₆₁ contributes to the cognitive deficits in AD by increasing the internalization of NMDAR from synaptic membrane surfaces.

Key words: Alzheimer disease, amyloid beta, NMDA receptor, protein tyrosine phosphatases, STEP, synaptic plasticity

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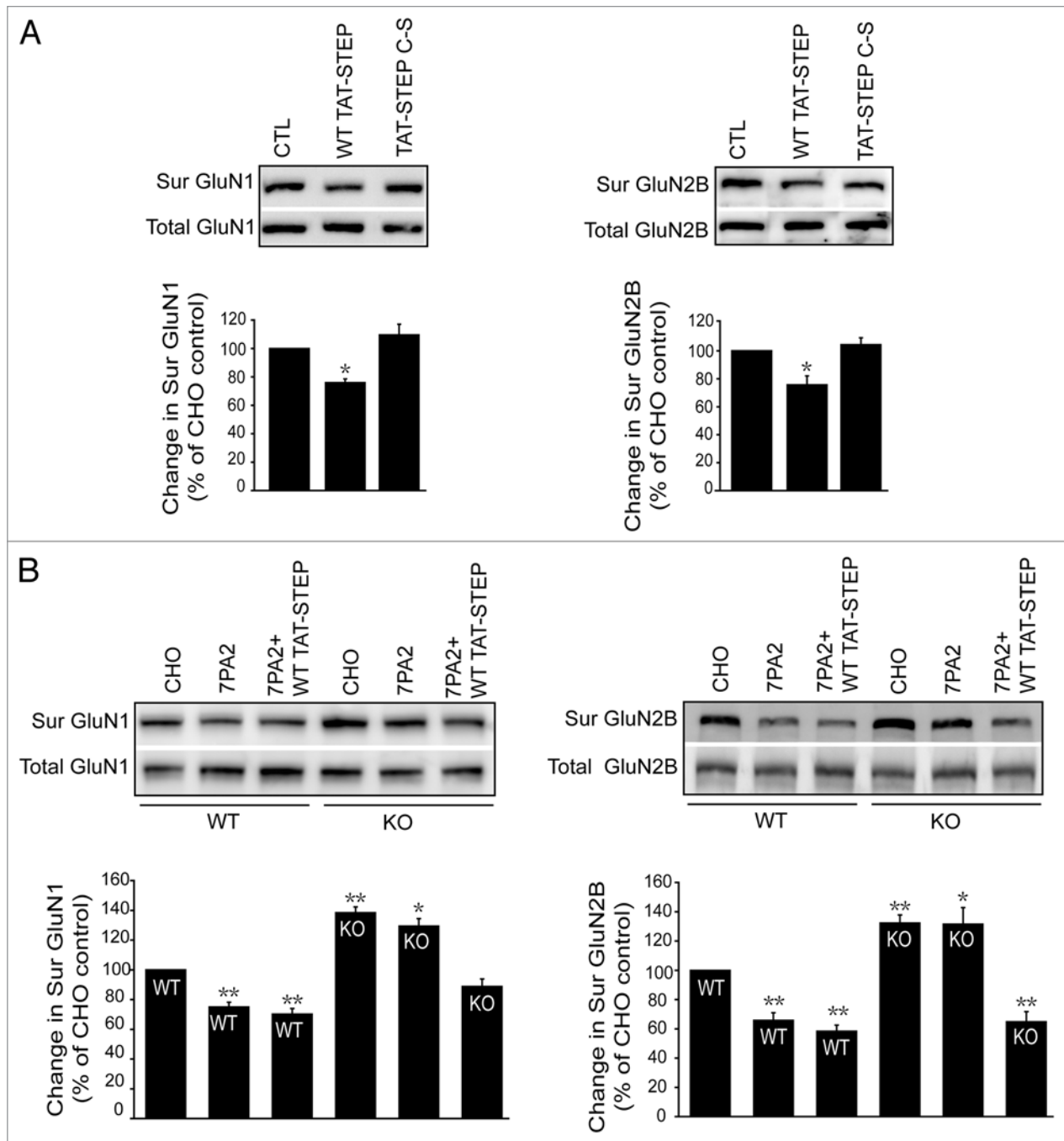


Figure 1. STEP activity regulates GluN1/GluN2B trafficking. (A) Surface biotinylation of wild-type cortical cultures after treatment with WT TAT-STEP or an inactive variant TAT-STEP C-S and probed for surface and total GluN1 and GluN2B with respective antibodies. Biotinylated receptors were normalized to total receptor level and then to loading control to determine percent change. Quantitation shows a significant decrease in surface GluN1 and GluN2B subunits in WT TAT-STEP treated, compared to TAT-STEP C-S or untreated control (* $p < 0.05$; one-way ANOVA with post hoc Tukey's test; $n = 3$). (B) Surface biotinylation of wild-type and STEP KO cortical cultures after treatment with CHO (control) or 7PA2-CM ($A\beta$ -enriched) medium in the presence or absence of WT TAT-STEP protein and probed for surface and total GluN1 and GluN2B with respective antibodies. Quantitation show a significant decrease in surface GluN1 and GluN2B subunits in wild-type cultures upon 7PA2-CM or 7PA2-CM + WT TAT-STEP treatment compared to CHO treated, whereas the effect of 7PA2-CM was absent in STEP KO cultures and was rescued in the presence of WT TAT-STEP protein (* $p < 0.05$, ** $p < 0.01$; one-way ANOVA with post hoc Tukey's test; $n = 4$).

STEP Activity Regulates the Expression of GluN1/GluN2B Subunits on Neuronal Surface

To clarify the role of STEP in GluN1/GluN2B endocytosis, we overexpressed STEP protein in wild-type (WT) cortical cultures by transducing WT TAT-STEP protein and examined surface levels of GluN1/GluN2B using biotinylation.^{4,10} Incubation of WT TAT-STEP protein (2 μ M; 1 hr) resulted in a significant decrease in surface GluN1 ($76.9 \pm 1.9\%$) and GluN2B ($75.0 \pm 5.7\%$) compared to the control (Fig. 1A). Incubation of a catalytically inactive STEP protein C³⁰⁰S (TAT-STEP C-S) failed to decrease surface levels of GluN1 ($108.0 \pm 6.9\%$) and GluN2B ($103.8 \pm 3.9\%$) (Fig. 1A). This result suggests that active STEP protein is required for internalization of GluN1 and GluN2B subunits from the neuronal surface membranes.

A β Treatment Fails to Induce NMDAR Endocytosis in STEP KO Cultures

To study the role of STEP in A β -mediated NMDAR endocytosis, we took advantage of STEP KO cultures.¹¹ We examined A β -mediated NMDAR endocytosis in STEP KO cultures using two complementary techniques: surface biotinylation and immunofluorescence as described previously.^{4,9} We used 7PA2-conditioned medium (7PA2-CM) as a source of A β . These cell secrete soluble amyloid-beta oligomers into the medium that were concentrated for use in these experiments.^{12,13} Cortical cultures derived from WT and STEP KO (18 DIV) were treated with 7PA2 (A β -enriched) or CHO (control) medium and GluN1 and GluN2B levels were determined by surface biotinylation. The surface GluN1 and GluN2B levels were significantly decreased in WT cultures after 7PA2-CM (A β -enriched) treatment compared to control (GluN1: $75.2 \pm 1.8\%$; GluN2B: $64.8 \pm 3.7\%$) (Fig. 1B). As previously shown, there was no change in the surface GluN2A levels upon 7PA2-CM treatment⁹ (data not shown). As expected, STEP KO cultures showed increase in basal levels of GluN1 and GluN2B compared to WT

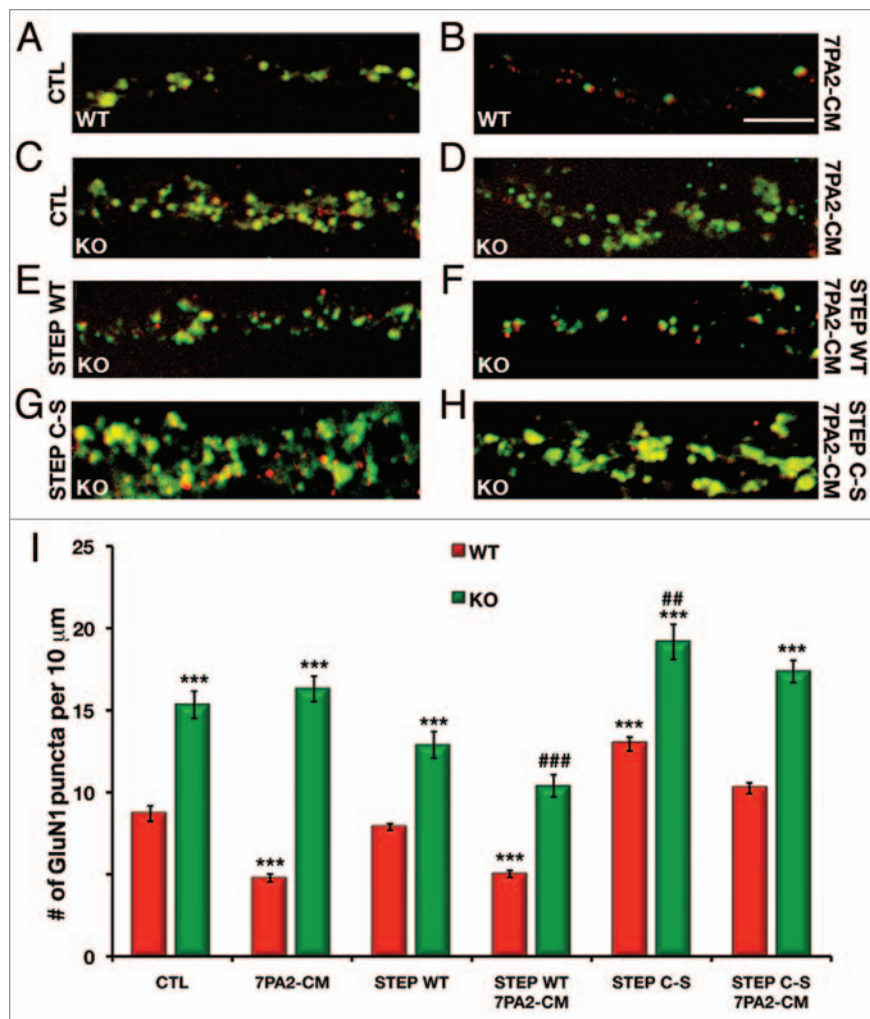


Figure 2. STEP KO cultures show no decrease in GluN1 surface levels after 7PA2-CM (A β -enriched) treatment. (A–D) Representative images from STEP WT and KO cortical cultures either untreated or treated with 7PA2-CM (A β -enriched) showing GluN1 surface staining (green) and the presynaptic marker synapsin I (red). (E and F) STEP KO cortical cultures pretreated with WT TAT-STEP alone or WT TAT-STEP followed by 7PA2-CM. (G and H) STEP KO cortical cultures pretreated with TAT-STEP C-S (inactive) alone or TAT-STEP C-S followed by 7PA2-CM. (I) Histogram showing the quantification of the surface GluN1 staining that colocalized with the presynaptic marker, synapsin I. STEP KO cultures showed significantly higher levels of surface GluN1 compared to WT controls. Treatment of WT cultures with 7PA2-CM resulted in a significant loss of GluN1; however, this was abolished in the STEP KO cultures. Restoration of STEP WT protein, but not inactive STEP C-S, rescued the 7PA2-CM-mediated endocytosis of GluN1 in the KO cultures (***) $p < 0.001$ as compared to WT control; ### $p < 0.001$ or ## $p < 0.01$ as compared to KO control, two-way ANOVA, $n = 4$ independent experiments, scale bar: 5 μ m).

(GluN1: $138.9 \pm 3.5\%$; GluN2B: $130.0 \pm 4.4\%$) and 7PA2 treatment failed to significantly decrease the surface GluN1 and GluN2B receptors (GluN1: $129.9 \pm 3.7\%$; GluN2B: $129.6 \pm 10.4\%$) (Fig. 1B). Preincubation with WT TAT-STEP protein during 7PA2-CM treatment rescued GluN1/GLUN2B internalization (GluN1: $89.2 \pm 3.3\%$; GluN2B: $63.7 \pm 5.6\%$) in STEP KO cultures compared to CHO controls (Fig. 1B).

Next, we examined the A β -mediated NMDAR endocytosis in WT and STEP KO cortical cultures using immunofluorescence to measure the colocalization of GluN1 with the presynaptic marker synapsin I.⁴ Treatment of neuronal cultures from WT mice with 7PA2-CM significantly decreased surface GluN1 staining (4.78 ± 0.24 puncta per 10 μ m, $p < 0.001$) as compared to untreated WT (8.69 ± 0.47 puncta per 10 μ m) cultures

(Fig. 2A and B). Untreated STEP KO cultures had significantly higher numbers of GluN1 puncta (15.33 ± 0.83 puncta per $10 \mu\text{m}$, $p < 0.001$) compared to WT cultures (Fig. 2A and C). A β -mediated endocytosis was abolished in STEP KO cultures (16.31 ± 0.78 puncta per $10 \mu\text{m}$, $p > 0.99$ as compared to KO control) (Fig. 2C and D).

We examined whether we could rescue A β -induced GluN1 endocytosis by restoring STEP protein to the KO cultures. The WT cortical cultures treated with WT TAT-STEP were similar to untreated WT cultures either in the presence or absence of 7PA2-CM (Fig. 2I). Treatment of KO cortical cultures with WT TAT-STEP reduced GluN1 surface expression (12.89 ± 0.81 puncta per $10 \mu\text{m}$, $p > 0.20$ as compared to KO control, Fig. 2E) and resulted in significant A β -induced GluN1 internalization (10.39 ± 0.67 puncta per $10 \mu\text{m}$, $p < 0.001$) as compared to both KO cultures and KO cultures treated with 7PA2-CM (Fig. 2F). The surface GluN1 levels in KO cultures treated with WT TAT-STEP followed by 7PA2-CM were not significantly different from WT cultures ($p > 0.74$), suggesting that restoration of WT STEP protein in KO cultures rescues A β -induced GluN1 endocytosis. In contrast, treatment of KO cortical cultures with inactive TAT-STEP C-S fusion protein resulted in a significant increase in surface expression of GluN1 receptors (19.17 ± 1.07 puncta per $10 \mu\text{m}$, $p < 0.002$

as compared to KO control, Fig. 2G) and the inactive STEP (C-S) fusion protein did not rescue A β -induced endocytosis of GluN1 subunits (17.36 ± 0.68 puncta per $10 \mu\text{m}$, $p > 0.99$) (Fig. 2H). The WT cultures treated with TAT-STEP C-S showed a significant increase in GluN1 surface expression and partially blocked the A β mediated GluN1 internalization (Fig. 2I).

In summary, these studies indicate that active STEP protein is required for A β -induced internalization of NMDARs. It also suggests a new therapeutic strategy by which inhibiting STEP activity could lead to the development of a new family of therapeutic agents in Alzheimer disease.

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