Reduction of Astrocytic Glutamate Transporter Contributes to Amyloid-Induced Microglial Pruning of Synapses

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Introduction: Astrocytes are the most abundant and heterogeneous type of glial cells in the brain. To determine how dysfunctional astrocyte glutamate transporters contribute to microglial pruning of glutamatergic synapses and memory deficiency induced by amyloid fibrils, we tested the hypothesis that amyloid fibrils impair astrocyte glutamate transporters, thus leading to an abnormal accumulation of extrasynaptic glutamate, which in turn contributes to microglial pruning of glutamatergic synapses and memory deficiency in the rodent models of Alzheimer's disease (AD).

Method: $A\beta_{1\text{-}40}$ fibrils were microinjected bilaterally into hippocampal CA1 areas in rats. In another cohorts, DL-threo-beta-benzyloxyaspartate (DL-TBOA), a specific inhibitor of glutamate transporter 1 (GLT1), or ceftriaxone (used to enhance GLT1 function) were also microinjected into hippocampal CA1 areas. Immunostaining and immunoblotting were performed to detect the expression of GLT1 in astrocytes. Complement C1q production and microglial pruning of glutamatergic synapses were assessed by 3D immunofluorescence imaging. Morris water maze test was performed to evaluate the cognitive function.

Results: We first noted a significant GLT1 reduction in the hippocampal astrocytes in rats injected with amyloid fibrils, indicating dysfunctional glutamate uptake (**Fig. 1**). Microinjection of ceftriaxone significantly attenuated C1q expression and endocytosis of vGluT1 within microglia, and improved behavioral performance in the modeled rats (**Fig. 2**). These results suggested that upregulation of GluT1 expression restored the synaptic microglial pruning and cognition impaired by amyloid fibrils. Meanwhile, in naïve rats, microinjection of 10 nmol of DL-TBOA substantially increased C1q expression, increased localization of vGluT1 within microglia, and impaired performance in the Morris water maze (**Fig. 3**). This suggests that dysfunctional GluT1, which likely leads to extracellular accumulation of glutamate, contributes to C1q-mediated microglial pruning of synapses and cognitive deficits induced by amyloid fibrils.

Conclusion: The present study demonstrates that reduction of astrocytic GLT1 contributes to C1q production and microglial pruning of glutamatergic synapses, hippocampal synaptic dysfunction and memory deficit in the rodent AD model. Our data highlight new aspects in our understanding of the pathogenesis of AD, and provides new approaches for identification and development of novel therapeutic targets for AD.

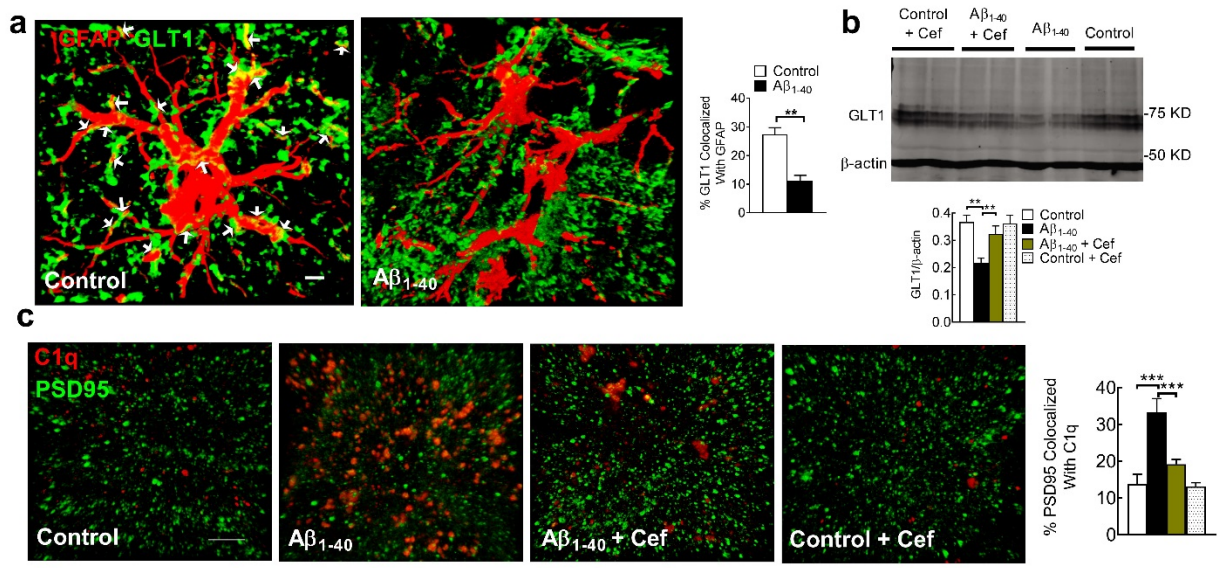

Fig. 1. Enhancement of astrocytic GLT1 function attenuated the C1q-mediated microglial pruning of synapses. Significantly decreased expression of GLT1 was observed in hippocampal CA1 in the modeled rats (***a,*** n = 7), which was recovered by microinjection of ceftriaxone (0.1 mg×7 days, ***b***, n=7); (***c***) Ceftriaxone also decreased the hippocampal C1q expression in astrocytes (***c,*** n = 6-7). The solid arrow indicated the internalization of vGluT1 within the microglia (Iba1), and the open arrow showed the overlay, not the internalization, of the immunosignals of Iba1 and vGluT1. **P<0.01; ***P<0.001. Data represent mean ± s.e.m

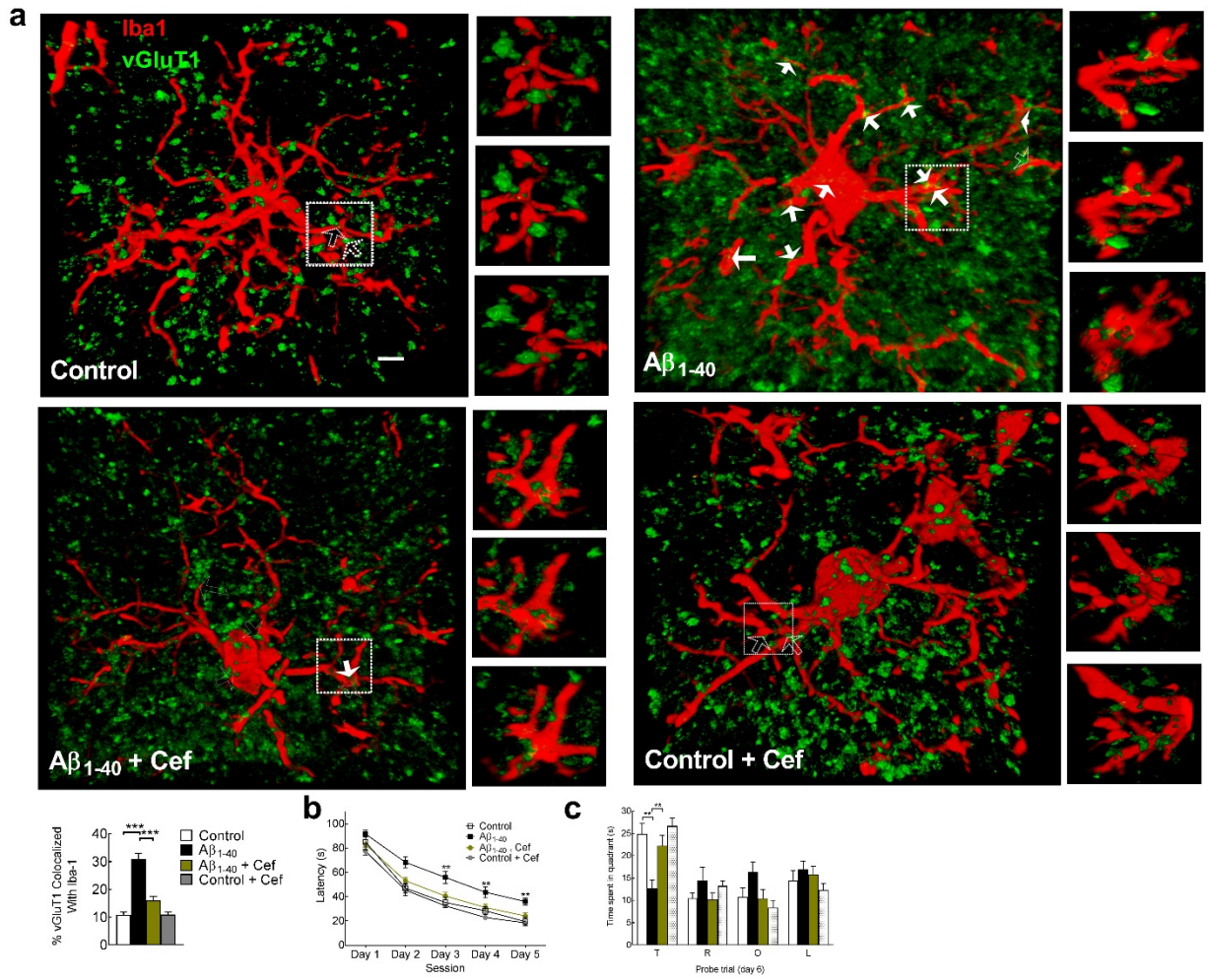

Fig. 2. Ceftriaxone decreased phagocytosis of vGluT1 by microglia (***a***, n = 4 rats; scale bar = 10 μ), decreased the escape latency (***b***) and increased the time in the target quadrant (***c***) in the modeled rats (n=10). An islet was rotated at about -45°, 0°, and 45° as described. The solid arrow indicated the internalization of vGluT1 within the microglia (Iba1), and the open arrow showed the overlay, not the internalization, of the immunosignals of Iba1 and vGluT1. **P<0.01; ***P<0.001. Data represent mean ± s.e.m.

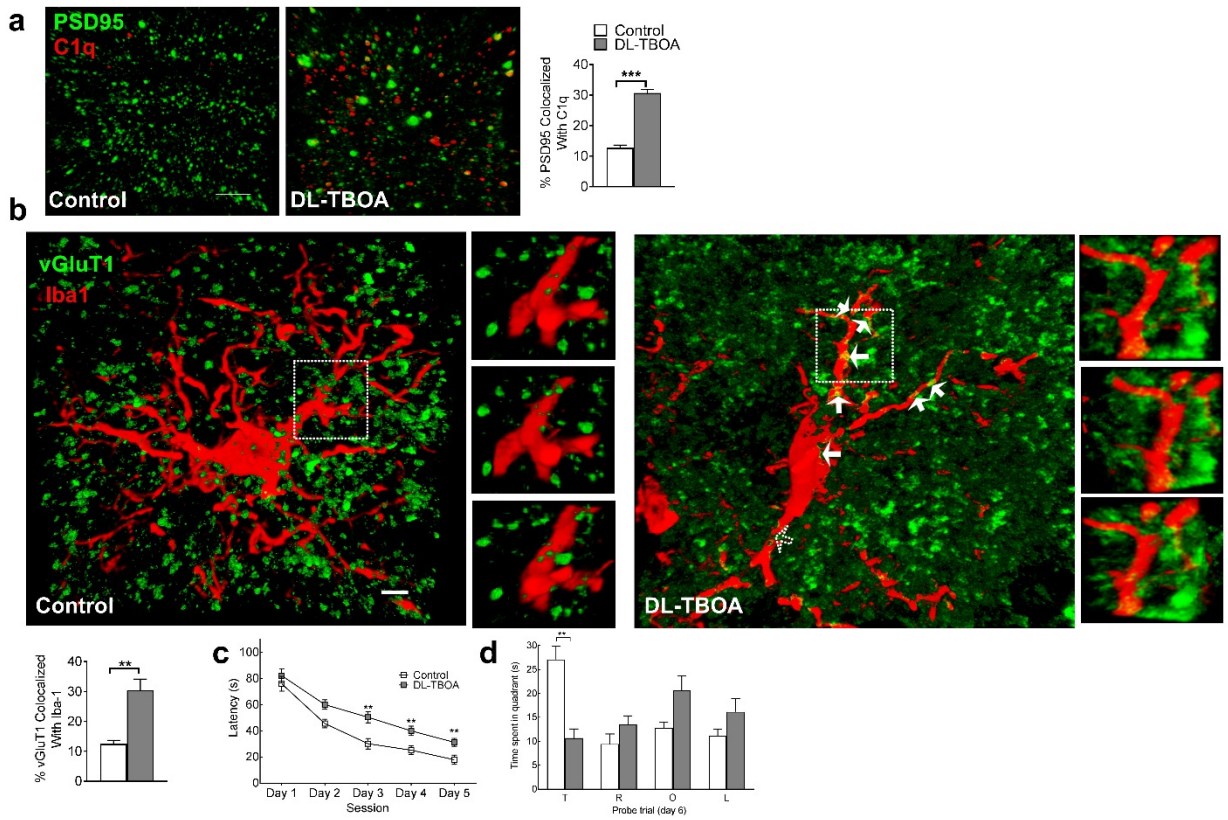

Fig 3. Suppression of astrocytic GLT1 by DL-TBOA induced microglial pruning of glutamatergic synapses. (**a**) DL-TBOA induced significant increase of C1q expression in the hippocampal CA1 synaptosome and its colocalization with PSD 95 in naïve rats (n = 4 sections from 5 rats); (***b-d***) DL-TBOA increased the phagocytosis of vGluT1 by microglia in the hippocampal CA1 (***b***, n = 4 rats per group; scale bar = 10 μ), increased escape latency (***c***, n = 10), and decreased the time in the target quadrant (***d***, n = 10) in the control rats. An islet was rotated at about -45°, 0°, and 45° as described. The solid arrow indicated the internalization of vGluT1 within the microglia (Iba1), and the open arrow showed the overlay, not the internalization, of the immunosignals of Iba1 and vGluT1. **, P<0.01; ***, P<0.001. Data represent mean ± s.e.m.