Amyloid Fibrils Induce Dysfunction of Hippocampal Glutamatergic Silent Synapses

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Introduction: Extensive neuroinflammation in Alzheimer's disease (AD) causes neuronal and synaptic loss, resulting in cognitive impairments. The molecular mechanisms underlying amyloid-induced synaptic dysfunction remain an area of interest. Our hypothesis is that accumulation of amyloid species results in the impairment of cytoskeleton protein (e.g., cofilin) and PSD scaffolding protein (e.g., PSD95), which decreases the distribution of GluR1 anchoring at PSD and leads to the dysfunction of glutamatergic synapses in the hippocampal CA1 and impairment of synaptic plasticity and cognition.

Methods: Rats injected with amyloid fibrils into hippocampal CA1 were used in this study. The minimal stimulation-based silent synapse recordings were performed on the hippocampal CA1 neurons and the activation of silent synapses was induced by pairing low-frequency electric stimuli. Immunoblotting was used to study the expression of GluR1. Whole-cell recording and Morris water maze were used to evaluate the synaptic and cognitive function in the rodent models.

Results: First, we noted that Amyloid impairs the unsilencing of glutamatergic silent hippocampal synapses. The percentage of silent synapses in the hippocampal CA1 in rats injected with $A\beta_{1-40}$ was lower than that in rats injected with saline (**Fig. 1**). Next, we found the reduction of the immunosignal of GluR1 was observed in the hippocampal synaptosome in rats injected with $A\beta_{1-40}$ (**Fig. 2**). Then, Amyloid impaired cytoskeletal actin dynamics and postsynaptic scaffolding protein. Microinjection of $A\beta_{1-40}$ substantially decreased the expression level of phosphorylated cofilin (**Fig. 3**). In addition, microinjection of $A\beta_{1-40}$ markedly decreased the expression level of PSD95 in the hippocampal CA1 synaptosome (**Fig. 4**). Microinjection of amyloid fibrils reduced the hippocampal glutamatergic strength and high-frequency electric stimuli-induced long term potentiation in hippocampal CA1 neurons, and impaired the performance in Morris water maze test in rats (**Fig. 5**).

Conclusion: This study demonstrated a reduction of hippocampal silent synapses, which failed to be activated by pairing low-frequency stimuli in the rodent model of AD. These findings may, at least partially, result from the impairment of the actin cytoskeleton and PSD scaffold proteins in the central neurons.

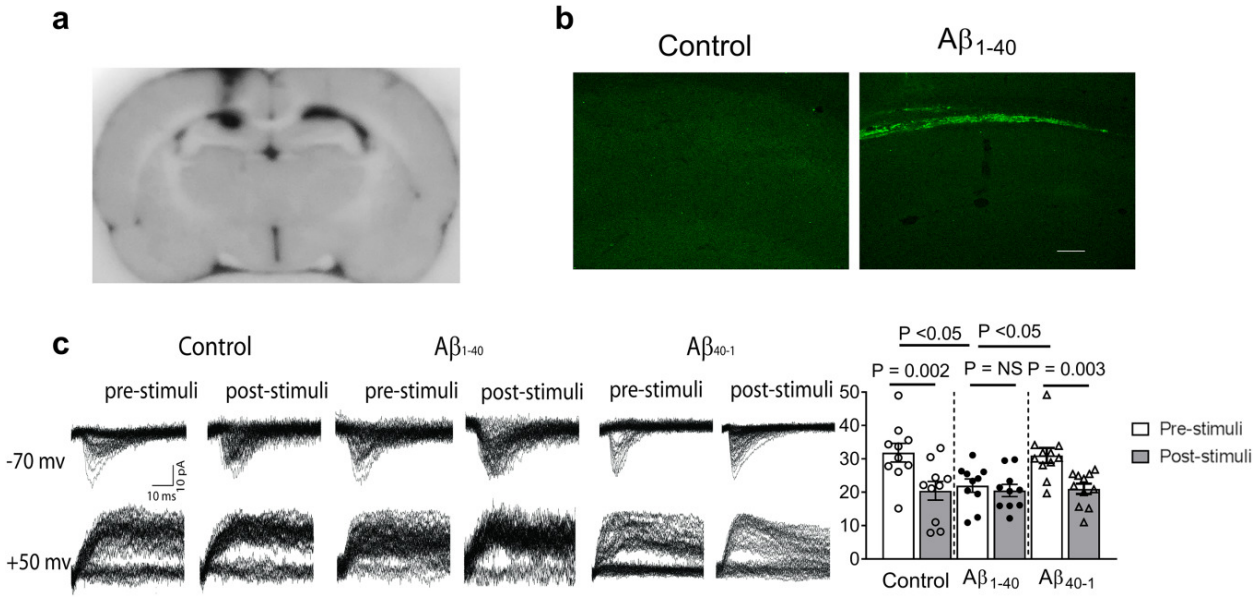

Fig. 1 Microinjection of $A\beta_{1-40}$ fibrils induced dysfunction of hippocampal silent synapses. (**a**) An India ink-marked microinjection site in the hippocampal CA1 area demonstrated the preciseness of the injection site. (**b**) Immunostaining images showed the existence of $A\beta_{1-40}$ in the rats injected with $A\beta_{1-40}$ after the completion of behavioral testing, which was clearly absent in the control rats. (**c**) EPSCs in the silent synapse appeared at baseline at a holding potential of +50 mV but not at –70 mV. After pairing with low-frequency electric stimuli, EPSCs appeared at –70 mV. The percentage of silent synapses among all recorded synapses was calculated as $1-Ln(F_{-70})/Ln(F_{+50})$, in which F_{-70} is the failure rate at -70 mV and F_{+50} is the failure rate at +50 mV. The percentage of silent synapse (within groups) was compared using paired t-test in the control group ($t = 4.515$, $DF = 9$, two-tailed $P = 0.002$), $A\beta_{1-40}$ group ($t = 0.72$, $DF = 9$, $P = 0.49$), and $A\beta_{40-1}$ group ($t = 3.98$, $DF = 10$, $P = 0.003$). Pre-stimuli were compared among the groups using one-way ANOVA ($F = 5.25$, $DF = 30$, two-tailed $P = 0.012$). Data represent mean ± s.e.m.

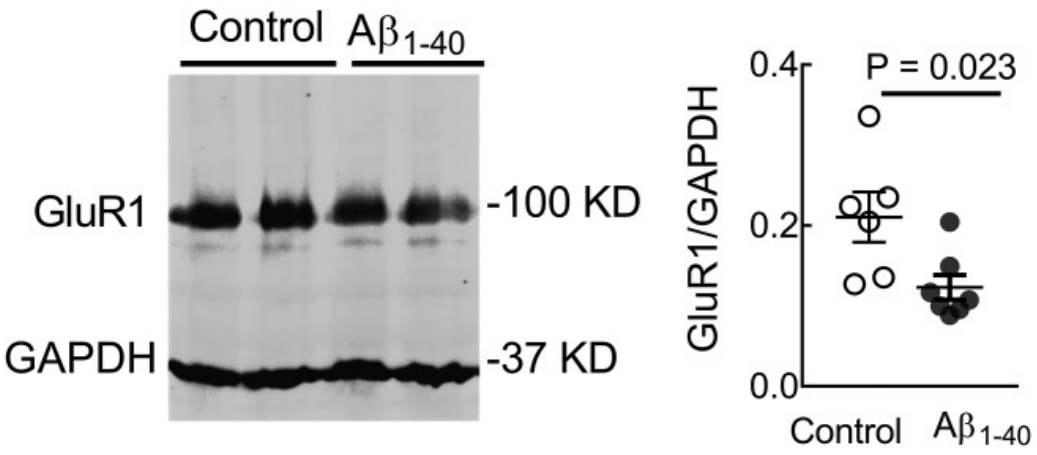

Fig. 2 Significantly decreased expression of AMPA receptor subunit GluR1 in the hippocampal CA1 synaptosomal preparation, which indicated a reduced distribution of GluR1 in glutamatergic synapses, in rats injected with $A\beta_{1\text{-}40}$ ($t = 2.63$, $DF = 11$, two-tailed $P = 0.023$). Data represent mean ± s.e.m.

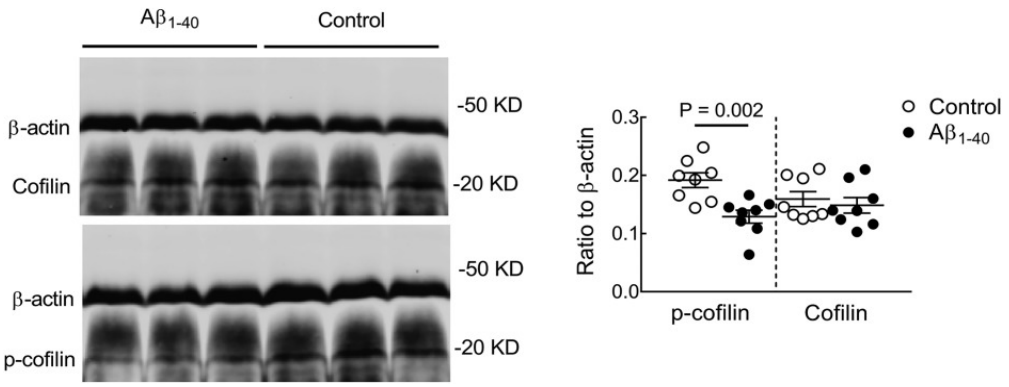

Fig. 3 Microinjection of $A\beta_{1\text{-}40}$ significantly decreased the expression of phosphorylated cofilin ($t = 3.74$, $DF = 14$, two-tailed $P = 0.002$), but not that of total cofilin ($t = 0.58$, $DF = 14$, two-tailed $P = 0.6$), in the hippocampal CA1 in rats injected with $A\beta_{1\text{-}40}$. These results indicated a potential dysfunction of actin cytoskeleton in hippocampal CA1 in the modeled rodents. Data represent mean ± s.e.m.

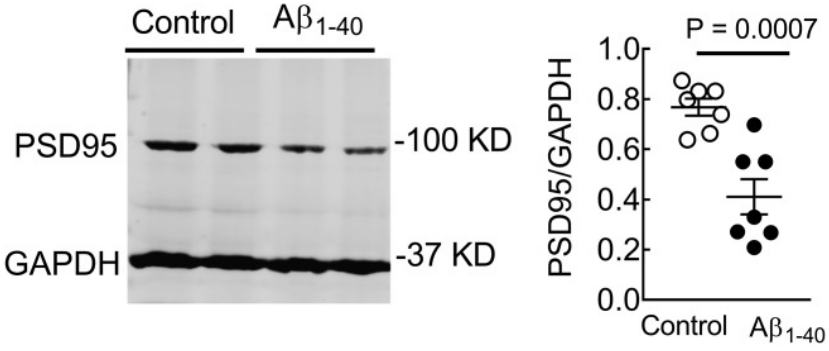

Fig. 4 Significantly decreased the expression of scaffolding protein PSD95 in hippocampal CA1 synaptosomal preparation in rats injected with amyloid fibrils (t = 4.56, DF = 12, two-tailed P = 0.0007). This potentially contributed to the dysfunction of hippocampal silent synapses in the rat injected with A□$_{1-40}$. Data represent mean ± s.e.m.

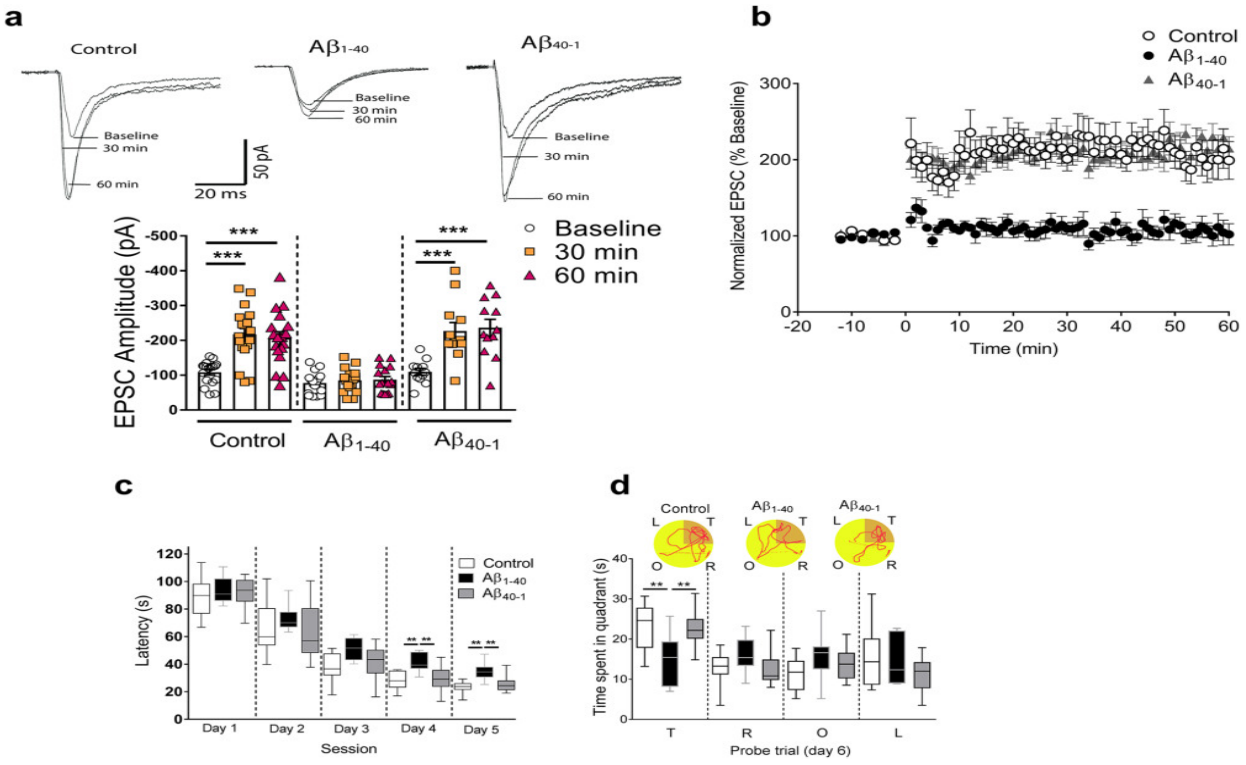

Fig. 5 Hippocampal injection of A$\beta_{1–40}$ fibrils impaired memory and glutamatergic synaptic plasticity. (a-b) Significantly impaired long-term potentiation (LTP) in the hippocampal CA1 neurons induced by microinjection of Aβ_{1-40}. LTP was induced by electric stimuli on the Schaffer collateral–commissural fibers at 100 Hz for 1 second. (a) The representative traces of EPSCs were presented to show the evoked EPSCs at baseline, 30, and 60 minutes after electric induction. Data were analyzed with repeated measures ANOVA. (a) Control group (n = 18, $F_{2,17}$ = 42.8, P<0.0001), A$\beta_{1–40}$ group (n = 16, $F_{2,15}$ = 0.53, P = 0.6), and Aβ_{40-1} group (n = 12, $F_{2,11}$ = 40.2, P<0.0001). (b) Time course of the amplitude of EPSCs in all three groups (b, n = 18, 16 and 12 neurons in each group, $F_{2,43}$ = 18.7, P<0.0001). (c-d) Significantly extended escape latency (c**,** n = 10 rats in each group, effect of group [$F_{2,27}$ = 7.71, P<0.002], effect of time [$F_{4,27}$ = 200.9, P<0.0001], interaction between group and time [P= 0.47]) and less time spent in the target quadrant (d, n = 10 rats in each group, $F_{2,27}$ = 6.67, P = 0.004) in rats microinjected with A$\beta_{1–40}$ fibrils but not Aβ40-1 fibrils nor artificial CSF (control). Representative path tracings in each quadrant during the probe trial on day 6 (b, T, target quadrant; R, right quadrant; O, opposite quadrant; L, left quadrant). **, P<0.01. Data represent mean ± s.e.m. For box-and-whiskers plots, the box extends from the 25th to 75th percentiles, a line within the box marks the median. Whiskers (error bars) above and below the box represent the minimum and maximum values.