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Amyloid fibrils induce dysfunction of hippocampal glutamatergic silent synapses

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Abstract

Silent glutamatergic synapses lacking functional AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors exist in several brain regions including the hippocampus. Their involvement in the dysfunction of hippocampal glutamatergic transmission in the setting of Alzheimer's disease (AD) is unknown. The present study demonstrated a decrease in the percentage of silent synapses in rats microinjected with amyloid fibrils ($A\beta_{1-40}$) into the hippocampal CA1. Also, pairing low-frequency electric stimuli failed to induce activation of the hippocampal silent synapses in the modeled rats. Immunoblotting studies revealed a decreased expression of GluR1 subunits in the hippocampal CA1 synaptosomal preparation, indicating a potential reduction in the GluR1 subunits anchoring in postsynaptic density in the modeled rats. We also noted a decreased expression of phosphorylated cofilin, which regulates the function of actin cytoskeleton and receptor trafficking, and reduced expression of the scaffolding protein PSD95 in the hippocampal CA1 synaptosome in rats injected with $A\beta_{1-40}$. Taken together, the present study illustrates dysfunction of hippocampal silent synapse in the rodent model of AD, which might result from the impairments of actin cytoskeleton and postsynaptic scaffolding proteins induced by amyloid fibrils.

Keywords

Amyloid fibrils; silent synapse; cofilin; PSD95

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. Silent glutamatergic synapses, which exhibit N-methyl-D-aspartate (NMDA) receptor -, but not AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptor -mediated synaptic currents, exist in several brain regions including the hippocampus (Liao et al., 1995; Nusser et al., 1998; Petralia et al., 1999). Neuronal activity recruits AMPA receptors to the postsynaptic site to

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activate silent synapses and increase the strength of synaptic transmission.(Isaac et al., 1995; Li and Sheng, 2003) Incorporation of inwardly rectifying and calcium-permeable GluR1-containing AMPA receptors at silent synapses serves as a critical step in the early phases of electrical stimuli-induced long-term potentiation (LTP) in the hippocampal CA1 pyramidal neurons (Morita et al., 2014). Induction of silent synapses by sustained inactivation of neural circuits enhanced the LTP magnitude at Schaffer collateral CA1 synapses (Arendt et al., 2013). Synaptic dysfunction in AD is characterized by inhibition of LTP, facilitation of long-term depression, and loss of synaptic AMPA and/or NMDA receptors (Hsieh et al., 2006; Ting et al., 2007). Currently, the adaptation of hippocampal silent synapses and the underlying mechanisms remain elusive in the setting of AD.

Distribution and immobilization of AMPA receptors in the postsynaptic membrane depends on several intracellular processes including receptor biosynthesis, intracellular sorting process, actin cytoskeleton-mediated membrane trafficking and scaffold protein-mediated anchoring, clustering process, and synaptic activity (Isaac et al., 1995; Kerr and Blanpied, 2012; Li and Sheng, 2003). The actin cytoskeleton system at the synapses plays a pivotal role in regulating the spine morphology, in anchoring/trafficking and in dynamic localization of AMPAR subunits (Anggono and Huganir, 2012; Kerr and Blanpied, 2012), thus contributing to the induction and maintenance of synaptic plasticity in central neurons (Cingolani and Goda, 2008; Yuen et al., 2010). Binding of dephosphorylated cofilin (the active form of cofilin) to filamentous actin (F-actin) results in actin severing and depolymerization, which negatively regulates the actin dynamics and synaptic plasticity (Cingolani and Goda, 2008; Yuen et al., 2010). Scaffold proteins in postsynaptic density (PSD) control the trafficking, anchoring, and clustering of glutamate receptors and adhesion molecules (Iasevoli et al., 2013). PSD95 is a major core scaffolding protein that anchors the glutamate receptors in PSD of excitatory synapses, regulates numerous aspects of synapse dynamics including synapse maturation and synaptic plasticity in central neurons (Elias and Nicoll, 2007; Kim and Sheng, 2004; Xu, 2011). Knockdown of PSD95 suppressed the activation of silent synapses and blocked the induction of LTP in developing superficial superior colliculus neurons (Zhao et al., 2013). In the present study, we were interested in the potential role that impairment of actin cytoskeleton and scaffold proteins plays in the dysfunction of hippocampal silent synapses in the rodent model of AD.

We used behavioral, electrophysiological, and molecular approaches to test the hypothesis 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.

Materials and Methods

1. Animals

The Animal Care and Use Committee of Cleveland Clinic approved all animal procedures which were performed in accordance with guidelines of the National Institutes of Health. Animals were housed the Institutional Biological Rodent Unit on a 14/10-hour light/dark cycle with water and food pellets available *ad libitum*. Adult male Sprague-Dawley rats

(weighing 200–250 g) were purchased from Charles River (Wilmington, MA, USA), and all experiments were performed during the light cycle. Behavioral testing was performed on 8th day after the hippocampal microinjection. The samples for morphological and immunoblotting studies were collected immediately after the behavioral testing. The tissues were sampled within a range of ~400 μ m around the injection site.

2. Microinjection of amyloid fibrils into the hippocampal CA1 area

Rats were anesthetized with sodium pentobarbital (45 mg/kg i.p.) and restrained in a stereotaxic apparatus (Bie et al., 2014). A β _{1–40} fibrils were formed as described previously (Chacon et al., 2004). A β _{1–40} fibrils (10 μ g/3 μ l), A β _{40–1} (10 μ g/3 μ l) or 3 μ l of artificial cerebrospinal fluid were injected stereotaxically and bilaterally into each hippocampus (anteroposterior: –3.5 mm, mediolateral: $\pm$ 2.0 mm, dorsoventral: –3.0 mm) (Paxinos and Watson, 1998) using a 10 μ l Hamilton syringe with a 27 G stainless steel needle at a rate of 0.5 μ l/min. This experimental model has been used for studying AD (Ahmed et al., 2010; Bie et al., 2014; Chacon et al., 2004; Shin et al., 1997; Wu et al., 2013a; Wu et al., 2013b). The accuracy of microinjection site was histologically verified afterward (Bie et al., 2014; Wu et al., 2013a; Wu et al., 2013b). We cannot absolutely exclude the contaminant effects from other amyloid species (potential residue of A β _{1–40} peptide and degradation of fibrils over time) in the present rodent model.

3. Morris water maze

The Morris water maze test was performed to investigate the memory function of rats (Bie et al., 2014; Wu et al., 2013a; Wu et al., 2013b). A total of 10 rats were randomly included in each group, with a breakdown of 5 rats per group for two separate rounds of testing. Testing was conducted in all groups at the same time of the day (9:00 AM to 12:00 noon). The experimental apparatus consisted of a circular pool (180 cm in diameter, 45 cm high). An invisible platform (15 cm in diameter, 35 cm high) was placed 1.5 cm below the surface of the water. The swimming path of the rat was recorded by a video camera and analyzed using EthoVision XT software (Noldus Information Technology). Each rat underwent four trials per day for five consecutive days. During each trial (memory acquisition), the animals were placed into the maze consecutively from four random points of the pool and were allowed to search for the platform for 120 seconds. If the animal did not find the platform within 120 s, they were gently guided to it. Rats were allowed to remain on the platform for 20 seconds. The latency for each trial was recorded for analysis. On Day 6, all rats were subjected to a probe trial (memory retrieval) in which the platform was removed, and each animal had 60 seconds to search the pool for the platform. All behavioral testing was performed by the same investigator blinded to the different treatment groups.

4. Hippocampal slice preparation and whole-cell recordings

For these studies, a different cohort of 24 rats was utilized (n = 8 per group). These rats underwent behavioral testing, as described before, but behavioral data were not included in the analysis. The electrophysiological recording was performed within 24 hours (0–1 day) after the conclusion of behavioral testing. Rat brain slices containing the hippocampal CA1 area were prepared, as previously described (Bie et al., 2010; Bie et al., 2009a; Bie et al., 2009b) after completing behavioral testing (Bie et al., 2014). The brain was quickly removed

and cut on a Vibratome in cold physiological saline to obtain coronal slices (300 μm thick) containing the hippocampus. A single slice was submerged in a shallow recording chamber and perfused with warm (35°C) physiological saline. Whole-cell voltage-clamp recordings from the CA1 area were taken using an Axopatch 200B amplifier (Molecular Devices) with 2–4 M Ω glass electrodes containing the internal solution (mM): cesium methanesulfonate, 125; NaCl, 5; MgCl₂, 1; EGTA (ethylene glycol tetraacetic acid), 0.5; Mg-ATP (ATP, Adenosine-5'-triphosphate), 2; Na₃-GTP (GTP, guanosine-5'-triphosphate), 0.1; HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10; pH 7.3; 290–300 mOsmol. A seal resistance of ≥ 2 G Ω and an access resistance of 15–20 M Ω were considered acceptable. The series resistance was optimally compensated by $\geq 70\%$ and constantly monitored throughout the experiments. The membrane potential was held at -70 mV, unless otherwise stated, throughout the experiment. Schaffer collateral commissural fibers were stimulated by ultrathin concentric bipolar electrodes (FHC Inc, Bowdoinham, ME), and the excitatory postsynaptic currents (EPSCs) were recorded in the presence of the gamma-aminobutyric acid receptor type A (GABA_A) receptor antagonist bicuculline (30 μM) in the CA1 area. The evoked EPSCs were filtered at 2 kHz, digitized at 10 kHz, and acquired and analyzed using Axograph X software. If synaptic transmission was stable ($<15\%$ change in EPSC amplitude over 15 minutes), long-term potentiation (LTP) was induced by a single high-frequency electric stimuli train (100 Hz for 1 second) (Bie et al., 2014; Wu et al., 2013a; Wu et al., 2013b). All electrophysiological experiments were performed at room temperature ($21 \pm 1^\circ\text{C}$).

5. Detection and activation of silent glutamatergic synapses

The minimal stimulation-based silent synapse recordings were performed on the hippocampal CA1 neurons (Liao et al., 1995). After obtaining a small EPSC at -70 mV, the intensity of the electric stimuli was reduced to the point that failures vs. successes of EPSCs could be clearly distinguished visually. For each cell, 100 traces were recorded at -70 mV and $+50$ mV, respectively, for two rounds. Only cells with relatively constant failure rates (changes $<10\%$) between rounds were included. The percentage of silent synapses among all recorded synapses was calculated as $1 - \text{Ln}(F_{-70}) / \text{Ln}(F_{+50})$, in which F_{-70} was the failure rate at -70 mV and F_{+50} was the failure rate at $+50$ mV, as rationalized previously (Isaac et al., 1995; Liao et al., 1995). The percentage of silent synapses were compared between the groups.

The activation of silent synapses was induced by pairing low-frequency electric stimuli (Liao et al., 1995). For the electric stimuli paradigm, synaptic plasticity was elicited by depolarizing the neuron to 0 mV and pairing low frequency presynaptic activity at 2 Hz for 120 pulses with constant stimulus intensity. This paradigm was widely applied to activate the silent synapses in hippocampal CA1 neurons (Liao et al., 1995).

6. Synaptosomal preparations and protein extraction

The protocol for preparing synaptosomes was based on previous reports (Bie et al., 2014; Wu et al., 2016). Hippocampal CA1 tissues from rats injected with saline or A β_{1-40} and were gently homogenized in ice-cold 0.32 M sucrose buffer at pH 7.4, and then centrifuged for 10 min at 1000 g (4°C). The supernatant was collected and centrifuged for 20 min at

10,000 g (4°C). The synaptosomal pellet was then resuspended in the lysis buffer (0.1% Triton X-100, 150 mM NaCl, 25 mM KCl, 10 mM Tris-HCl, pH 7.4, with protease inhibitors) at 4°C for 10 min. For total protein preparations (Bie et al., 2014), the hippocampal CA1 tissues from the saline- and amyloid fibrils-treated rats were homogenized on ice for 10 min in the lysis buffer containing 50 mM Tris-Cl, 150 mM NaCl, 0.02 mM NaN₂, 100 µg/ml phenylmethyl sulfonyl fluoride, 1 µg/ml aprotinin, and 1% Triton X-100. The lysates were centrifuged at 14,000 rpm for 10 min at 4 °C, and the supernatant was used for SDS-polyacrylamide gel electrophoresis. Protein concentrations were determined by using the Bio-Rad (Hercules, CA) protein assay kit.

7. Immunoblotting

The protein samples were subjected to 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by immunoblot analysis. The blots were incubated overnight at 4 °C with a rabbit polyclonal anti-cofilin antibody (1:1000; Millipore), polyclonal anti-p-cofilin antibody (1:1000; Millipore), mouse monoclonal anti-PSD95 (1:1000; Cell Signaling Technology), monoclonal anti-GluR1 antibody (1:1000; Cell Signaling Technology, Inc, Danvers, MA, USA) or monoclonal anti-GAPDH antibody (1:500; Santa Cruz Biotechnology Inc). The membranes were washed extensively and then incubated with horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG antibody (1:10,000; Jackson ImmunoResearch Laboratories Inc). The immunoreactivity was detected using enhanced chemiluminescence (ECL Advance Kit; Amersham Biosciences). The intensity of the bands was captured digitally and analyzed quantitatively using ImageJ software. The immunoreactivity of target proteins was normalized to that of GAPDH.

8. Compounds and data analysis

Aβ peptide consisting of residues 1–40 of the human wild type sequence (Aβ_{1–40}) and reverse sequence peptide, Aβ_{40–1} was purchased from Bachem (Torrance, CA, USA). D-2-amino-5-phosphonopentanoate, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline, bicuculline, and other chemicals were purchased from Sigma Aldrich (St Louis, MO, USA) or Tocris (Ellisville, MO, USA).

Normality was tested using the Shapiro-Wilk test. For electrophysiological and behavioral analysis, the data were compared with two-way ANOVA. The data from the histological study, western blot, and other studies were analyzed using Student's *t*-test, one-way ANOVA test. *Post hoc* analyses were performed using Student-Newman-Keuls test. All statistical analyses were performed with BMDP statistical software (Statistical Solutions, Saugus, MA). Data were expressed as means ± SEM or as box-and-whiskers plots, with 25th to 75th percentiles, bars represent maximal and minimal values. For all tests, a two-tailed *P* < 0.05 was considered statistically significant.

Results

1. Amyloid impairs the unsilencing of glutamatergic silent hippocampal synapses

The accuracy of the microinjection was histologically verified by using an India ink to demonstrate the preciseness of the injection site in the hippocampal CA1 area (Fig 1a). In

addition, we performed immunostaining to demonstrate the existence of A β _{1–40} in the rats injected with A β _{1–40} after the completion of behavioral testing.

Next, we determined the percentage of silent glutamatergic synapses in hippocampal CA1 neurons using minimal stimulation-based recordings of EPSCs at –70 mV and +50 mV respectively (Liao et al., 1995). As shown in Fig. 1c, the percentage of silent synapses in the hippocampal CA1 in rats injected with A β _{1–40} was lower than that in rats injected with saline. Microinjection of A β _{40–1} peptide did not alter the percentage of glutamatergic silent synapses in hippocampal CA1 neurons (Fig. 1c). In addition, pairing low-frequency electric stimuli decreased the percentage of silent synapses in hippocampal CA1 neurons in rats injected with saline or A β _{40–1} peptide, but not in rats injected with A β _{1–40}. Together, these results demonstrated the reduction of glutamatergic silent synapses and the failure of activation by pairing low-frequency electric stimuli in hippocampal CA1 neurons in the rodent model of AD.

We next examined the expression of glutamate receptor subunit GluR1 in the synaptosomal preparation of the hippocampal CA1 in rats. As shown in Fig. 2, reduction of the immunosignal of GluR1 was observed in the hippocampal synaptosome in rats injected with A β _{1–40}, which indicated a potential reduction of GluR1 anchoring at PSD and may account for the failed activation of silent synapses by pairing low-frequency electric stimuli in the modeled rodents.

2. Amyloid impaired cytoskeletal actin dynamics and postsynaptic scaffolding protein

Previous studies have established that phosphorylation of cofilin serves as an important factor that regulates the trafficking and synaptic transports of AMPA receptor subunits in central neurons (Bernstein and Bamberg, 2010). We performed immunoblotting to detect the expression of total and phosphorylated cofilin in hippocampal CA1 in the rats injected with A β _{1–40} fibrils or saline. We found that microinjection of A β _{1–40} substantially decreased the expression level of phosphorylated cofilin (Fig. 3). These results suggested that the impaired function of the cytoskeleton might contribute to the deficit of the hippocampal silent synapses in the modeled rodents.

In addition, we detected the expression of scaffolding protein PSD95 in the hippocampal synaptosomal preparation in control and modeled rats. As shown in Fig. 4, microinjection of A β _{1–40} markedly decreased the expression level of PSD95 in the hippocampal CA1 synaptosome. These results taken together suggested that structural deficits of the glutamatergic synapse, e.g., impaired function of the cytoskeleton and loss of scaffolding protein and synaptic anchoring of receptor subunit GluR1, might contribute to dysfunction of the hippocampal silent synapses in the modeled rodents.

3. Microinjection of amyloid fibrils induced hippocampal glutamatergic dysfunction and memory deficiency

Considering the potential role of activation of silent synapses in the maintenance of synaptic plasticity in central neurons (Choquet and Triller, 2013), we next evaluated the function of hippocampal glutamatergic synaptic plasticity and cognition in the rats injected with amyloid fibrils. Consistent with our previous reports (Bie et al., 2014; Wu et al., 2013a; Wu

et al., 2013b), as shown in Fig. 5a–b, high frequency electric stimuli on the Schaffer collateral–commissural fibers induced potentiation of glutamatergic synaptic transmission in hippocampal CA1 neurons in rats injected with saline or A β _{40–1} peptide and injection of A β _{1–40} attenuated the intensity of LTP induced by high frequency electric stimuli was attenuated in the hippocampus CA1 neurons. Furthermore, Morris water maze test was performed to detect the cognitive behavioral performance in the rats. We found that microinjection of A β _{1–40}, but not A β _{40–1}, into the hippocampal CA1 significantly extended the escape latency (Fig. 5c) and decreased the time in the target quadrant (Fig. 5d) in the Morris water maze test (n = 10 per group, P<0.01), indicating an impaired cognitive function in the modeled rats. These results demonstrated that administration of A β _{1–40} into the hippocampal CA1 impaired the hippocampal glutamatergic synaptic plasticity and cognitive function in rodents.

Discussion

Silent synapses lacking AMPA receptors exist in several brain regions including the hippocampus (Liao et al., 1995; Nusser et al., 1998; Petralia et al., 1999), and altered function of silent synapses occurs in a number of physiological and pathological conditions, including addiction (Dong and Nestler, 2014; Huang et al., 2009; Ma et al., 2014), memory deficits (Geinisman, 2000) and several brain disorders (Hanse et al., 2013). Previous studies demonstrated that artificial manipulation to correlate the pre- and postsynaptic activity, e.g., pairing low-frequency electric stimuli (Ma et al., 2014) and classical conditioning (Keifer and Houk, 2011), may induce synaptic incorporation of the calcium-permeable AMPAR subunit GluR1 resulting in an activation of silent synapses and the enhanced synaptic plasticity (Hanse et al., 2013). Activation of silent synapses lacking AMPA receptors appears to be one of the principal postsynaptic mechanisms underlying the induction and expression of LTP induced by pairing low-frequency electric stimuli in the hippocampal CA1 neurons (Choquet and Triller, 2013; Liao et al., 1995).

Abnormally increased or decreased AMPA receptors in silent synapses may lead to faulty neural connectivity and immature spine morphology and may contribute to the pathogenesis of neurodevelopmental disorders, such as autism spectrum disorders and intellectual disability (Penzes et al., 2011). It was reported that A β promoted the removal of synaptic AMPA and NMDA receptors in the hippocampal neurons resulting in synaptic dysfunction (Hsieh et al., 2006; Ting et al., 2007). Based on these previous findings, we also noted in the present study a substantial reduction of the percentage of silent synapses in the hippocampal CA1 neurons in rats injected with A β _{1–40} species, and the failure to activate the hippocampal silent synapses by pairing of low-frequency electric stimuli in the modeled rodents. We also observed a reduction of GluR1 in the hippocampal synaptosome, which may underlie the failed activation of silent synapses by pairing low-frequency electric stimuli in the modeled rodents. These observations may explain, at least partially, the impairment of hippocampal glutamatergic synaptic plasticity noted in the rodent AD models.

The actin cytoskeleton plays a critical role in AMPA receptor membrane trafficking, thus modulating synaptic plasticity and memory in the physiological and pathological settings

(Yao et al., 2006). The actin depolymerizing factor (ADF)/cofilin family of actin-associated proteins regulates the dynamics of the actin cytoskeleton through their filament-severing and monomer-binding activities (Bernstein and Bamburg, 2010), possibly modulating the spine size and morphology (Hotulainen and Hoogenraad, 2010) and membrane trafficking of AMPA receptor subunits (Gu et al., 2010). ADF/cofilin is inactivated by phosphorylation of its serine-3 (Ser3) residue by LIM kinases and activated by dephosphorylation by specific phosphatases (Aizawa et al., 2001). A β oligomers appear to disturb the activity of p21-activated kinase-cofilin signaling, which contributes to the cognitive deficiency in AD (Zhao et al., 2006). A recent study reported that the suppression of glutamate receptor surface expression and synaptic function in frontal cortical neurons was correlated with the reduction of phosphorylated cofilin in a mouse model of familial AD (Deng et al., 2016), although others have noted an increased cofilin1 phosphorylation in the AD mouse (APP/PS1) model (Barone et al., 2014). It was also reported that injection of a cofilin dephosphorylation inhibitory peptide to familial AD mouse models resulted in a partial rescue of the surface expression of glutamate receptor subunits and cognitive capacity (Deng et al., 2016). Based on these previous studies, we found in the present study a reduction of phosphorylated cofilin, which coincided with the reduced synaptosomal GluR1, and resulted in impairment of the glutamatergic synaptic plasticity and cognition in rats injected with amyloid fibrils. The reduction of phosphorylated cofilin, considering its function to regulate actin cytoskeleton and synaptic plasticity, appears to contribute to the decreased expression of GluR1 in hippocampal synaptosome and dysfunction of silent synapses in the rodent of AD.

As eloquently stated by Choquet and Triller “Central synapse is a multiscale dynamic organelle whose function is intimately linked to movement of its individual components in space and time” (Choquet and Triller, 2013). PSD scaffold proteins (e.g., PSD95, Shank, GKAP, SAP70) regulate the trafficking, anchoring, and clustering of glutamate receptors and adhesion molecules, which is considered crucial in the modulation of synaptic plasticity and brain functions (Iasevoli et al., 2013). For instance, PSD95 can be recruited by the trans-synaptic adhesive molecules and promote the membrane diffusible AMPA receptors trapped in the synaptic cleft (Choquet and Triller, 2013) and GluR1 subunit membrane trafficking in the central neurons (Swayze et al., 2004). Knockdown of PSD95 decreased the postsynaptic AMPA and NMDA receptors and blocked the induction of LTP in central neurons (Zhao et al., 2013). Treatment with low concentration of A β induced early and transient (16–72 h) increase of synaptophysin and PSD95, followed by a decrease coincident with neuronal death (7d) in the organotypic hippocampal cultures (Merlo et al., 2016). Reduced expression of PSD95 was reported in the brain of rodent model of AD (Savioz et al., 2014). It was also reported that treatment with retinoid X receptor agonist bexarotene improved the remote memory stabilization in fear conditioning and olfactory cross habituation, which was correlated with significant increases in the expression of PSD95 in 5XFAD mice (Mariani et al., 2017). Based on these findings, we further identified that a reduction of synaptosomal PSD95 expression corresponded with the reduction of synaptic GluR1 and dysfunction of silent synapses in the hippocampal CA1 in rats injected with amyloid fibrils. Given the potential role of PSD95 in regulating AMPA receptor subunits trafficking and synaptic plasticity, the decreased expression of PSD95 may be implicated in the reduced expression

of GluR1 in hippocampal synaptosome and dysfunction of silent synapses in the rodent AD model.

In conclusion, the present 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.

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Fig. 1.

Microinjection of Aβ₁₋₄₀ 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 Aβ₁₋₄₀ in the rats injected with Aβ₁₋₄₀ 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 $P = 0.002$), Aβ₁₋₄₀ group ($t = 0.72$, $DF = 9$, $P = 0.49$), and Aβ₄₀₋₁ 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 $\pm$ 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 Aβ₁₋₄₀ ($t = 2.63$, $DF = 11$, two-tailed $P = 0.023$). Data represent mean $\pm$ s.e.m.

Fig. 3.

Microinjection of $A\beta_{1-40}$ 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 $A\beta_{1-40}$. These results indicated a potential dysfunction of actin cytoskeleton in hippocampal CA1 in the modeled rodents. Data represent mean $\pm$ 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 Aβ₁₋₄₀. Data represent mean ± s.e.m.

Fig. 5.

Hippocampal injection of Aβ₁₋₄₀ fibrils impaired memory and glutamatergic synaptic plasticity. (a–b) Significantly impaired long-term potentiation (LTP) in the hippocampal CA1 neurons induced by microinjection of Aβ₁₋₄₀. 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, $F_{2,17} = 42.8$, $P < 0.0001$), Aβ₁₋₄₀ group (n = 16, $F_{2,15} = 0.53$, $P = 0.6$), and Aβ₄₀₋₁ 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 Aβ₁₋₄₀ 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.