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Supplemental Information

Soluble Aβ Oligomers Impair Dipolar Heterodendritic Plasticity by Activation of mGluR in the Hippocampal CA1 Region

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Transparent Methods

Animals

The Harvard Medical School Standard Committee on Animals approved all experiments involving mice used for electrophysiology. All mice (male and female, 6~8 weeks old) contained a mixed background of C57Bl/6 and 129. Animals were housed in a temperature-controlled room on a 12-h light/12-h dark cycle and had ad libitum access to food and water.

Cellular Aβ preparations

Secreted human Aβ peptides were collected and prepared from the conditioned media (CM) of a CHO cell line (7PA2) that stably expresses human APP751 containing the V717F AD mutation (Podlisny et al., 1995) Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM L-glutamine, and 200 mg/ml G418 for selection. Upon reaching ~95% confluency, the cells were washed and cultured overnight (~15 h) in serum-free medium. CM was collected, spun at 1500 × g to remove dead cells and debris, and stored at 4°C. The CM was concentrated 10-fold with a YM-3 Centricon filter (Walsh et al., 2005). Aliquots of concentrated 7PA2 CM were stored at −80°C.

Preparation of Aβ isolated from AD cortex

Aβ from TBS extract of human AD cortical tissue was prepared as previously described (Shankar et al., 2008). While the TBS extract from non-AD human cortical tissue as a control brain TBS extract. Briefly, frozen human temporal or frontal cortices containing white and grey matter were weighed. Freshly prepared, ice cold Tris-buffered saline (TBS) consisting of 20 mM Tris-HCl, 150 mM NaCl, pH 7.4 was added to the frozen cortex at 4:1 (TBS volume: brain) and homogenized with 25 strokes at a setting of 10 on a mechanical Dounce homogenizer. The homogenate was spun at 175,000 g in a TLA100.2 rotor on a Beckman TL 100. The supernate (called TBS extract) was aliquoted and stored at −80 °C.

Hippocampal slice preparation

Mice (C57BL/6 × 129) were euthanized with Isoflurane at 8~10 wk of age. Brains was quickly removed and submerged in ice-cold oxygenated sucrose-replaced artificial cerebrospinal fluid (ACSF) cutting solution (206 mM sucrose, 2 mM KCl, 2 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 10 mM D-glucose, pH 7.4, 315 mOsm. Transverse slices (350 μm thickness) from the middle portion of each hippocampus were cut with a vibroslicer. After dissection, slices were incubated in ACSF that contained the following (in mM): 124 NaCl, 2 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose, pH 7.4, 310 mOsm, in which they were allowed to recover for at least 90 min before recording. A single slice was then transferred to the recording chamber and submerged beneath continuously perfusing ACSF that had been saturated with 95% O_2 and 5% CO_2. Slices were incubated in the recording chamber for 20 min before stimulation under room temperature (~26°C).

Electrophysiological recordings

We used standard procedures to record field excitatory postsynaptic potentials (fEPSP) in the CA1 region of the hippocampus. A bipolar stimulating electrode (FHC Inc., Bowdoin, ME) was placed in the Schaffer collaterals to deliver test and conditioning stimuli. Two borosilicate glass recording electrodes filled with ACSF were positioned in stratum radiatum (apical dendrites) and stratum oriens (basal dendrites) of CA1, 200~300 μm from the stimulating electrode. fEPSP in the CA1 region were induced by test stimuli at 0.05 Hz with an intensity that elicited a fEPSP amplitude 40-50% of maximum. Test responses were recorded for 30-60 min prior to beginning the experiment to assure stability of the response. Once a stable test response was attained, experimental treatments (Aβ oligomers, and/or other compounds) were added to the 10 mL ACSF perfusate, and a baseline was recorded for an additional 30 min. To induce LTP, two consecutive trains (1 s) of stimuli at 100 Hz separated by 20 s were applied to the slices, a protocol that induced LTP lasting approximately 1.5 hr in wild-type mice of this genetic background. Another LTP protocols were 10 Hz bursts stimulation consist of 10 pulses 10Hz bursts, 10 trains separated by 10 sec (intensive)

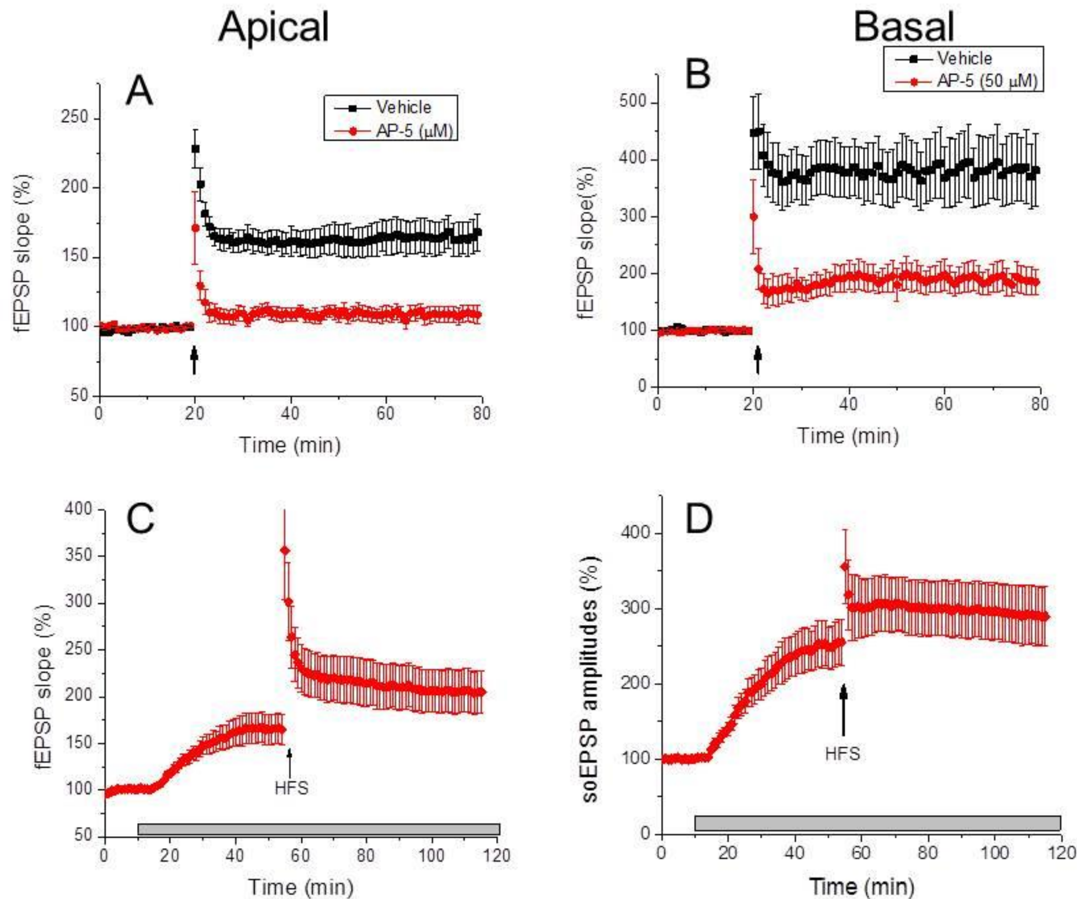
or 30 sec (spaced). To induce LTD, 300 pulses were delivered at 1 Hz. The field potentials were amplified 100x using an Axon Instruments 200B amplifier and digitized with Digidata 1322A. Data were sampled at 10 kHz and filtered at 2 kHz. Traces were obtained by pClamp 9.2 and analyzed using the Clampfit 9.2 program. LTP and LTD values reported throughout were measured at 60 min after the conditioning stimulus unless stated otherwise. In this study, "n" indicates the brain slice number. Paired-pulse responses were monitored from 20 to 200 ms inter-stimulus intervals. The facilitation ratio was calculated as fEPSP2 slope/fEPSP1 slope. Two-tailed Student's t-test and one-way analysis of variance (ANOVA) were used to determine statistical significance.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
DL-AP-5	Tocris Bioscience	**Cat. No. 3693/10**
SIB 1757	Tocris Bioscience	**Cat. No. 1215/10**
Philanthotoxin 74	Tocris Bioscience	**Cat. No. 2770/1**
CGP 35348	Tocris Bioscience	**Cat. No. 1245/10**
ZD 7288	Tocris Bioscience	**Cat. No. 1000/10**
Experimental Models: Organisms/Strains		
Mouse: C57BL/6J wild-type	The Jackson Laboratory	Stock No: 000664 | Black 6

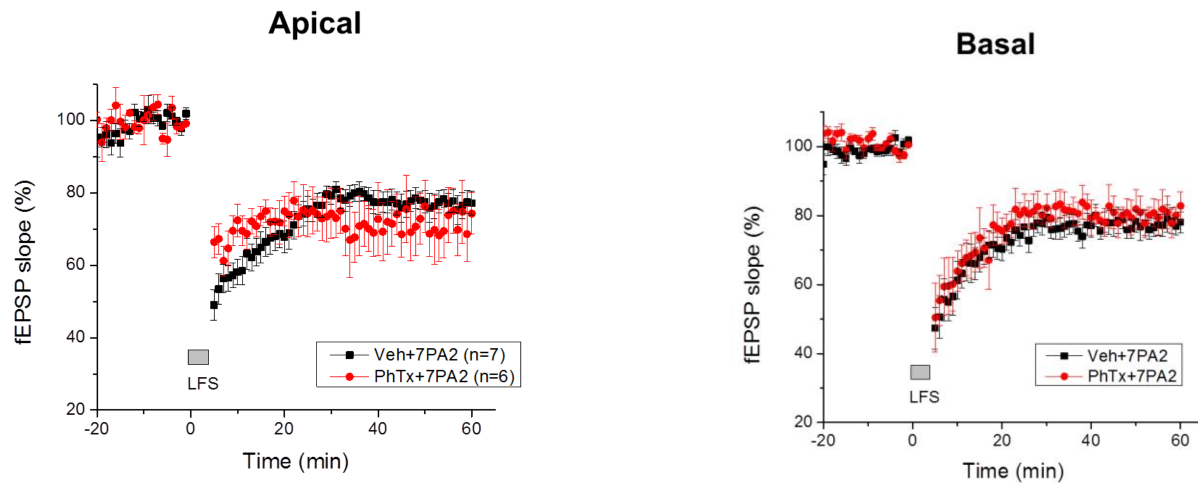
Supplemental Figures

Figure S1. (Related to Fig. 2.)



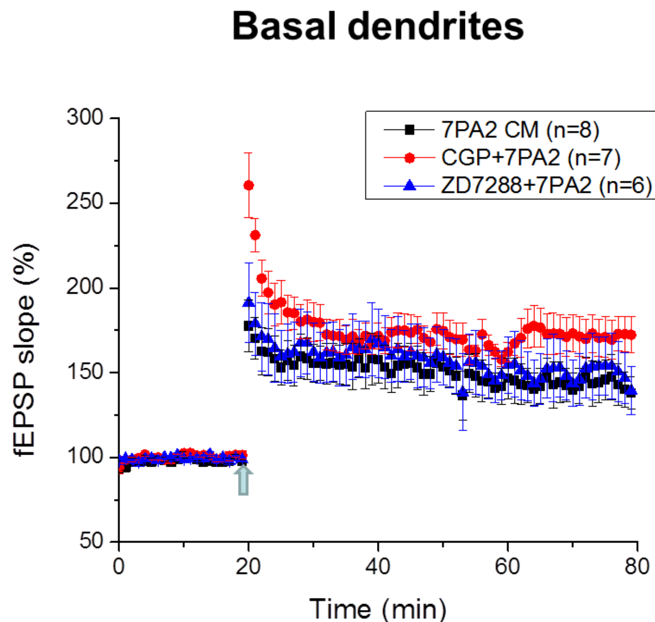
The cell-wide heterosynaptic basal dendritic LTP is NMDAR-dependent, similar to the homosynaptic (apical) LTP. The HFS induced LTP could be blocked by NMDA receptor antagonist, AP-5 (50 μM) recording from both apical (A) and basal (B) dendrites. Similarly, activation of NMDA receptors by removing the Mg^{2+} from the perfusate prevent HFS induced LTP in both apical (C) and basal (D) dendrites. Grey bar represent the Mg^{2+} free ACSF.

Figure S2. (Related to Fig. 3.)



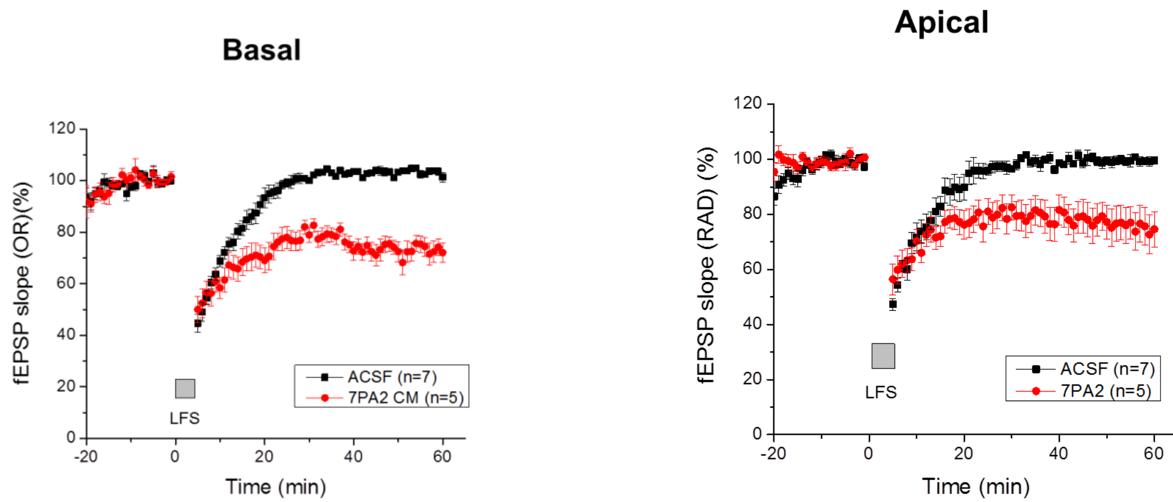
Soluble Aβ oligomers facilitated hippocampal LTD in both apical (A) and basal (B) dendrites are not require for calcium-permeable AMPA receptor.

Figure S3. (Related to Fig. 5.)



Soluble Aβ oligomers impaired hippocampal sapced-10 Hz LTP in basal dendrites are not involve in $GABA_B$ receptors and H-channel. As the $GABA_B$ receptor selective antagonist, CGP 35348 (10 μM) or H-channels blocker (ZD7288, 5 μM) failed to prevent 7PA2 CM impaired LTP.

Figure S4. (Related to Fig. 6.)



Both the homosynaptic basal dendritic LTD (left) and heterosynaptic apical LTD (right) were facilitated by 7PA2 CM treatment upon stimulation in stratum oriens.