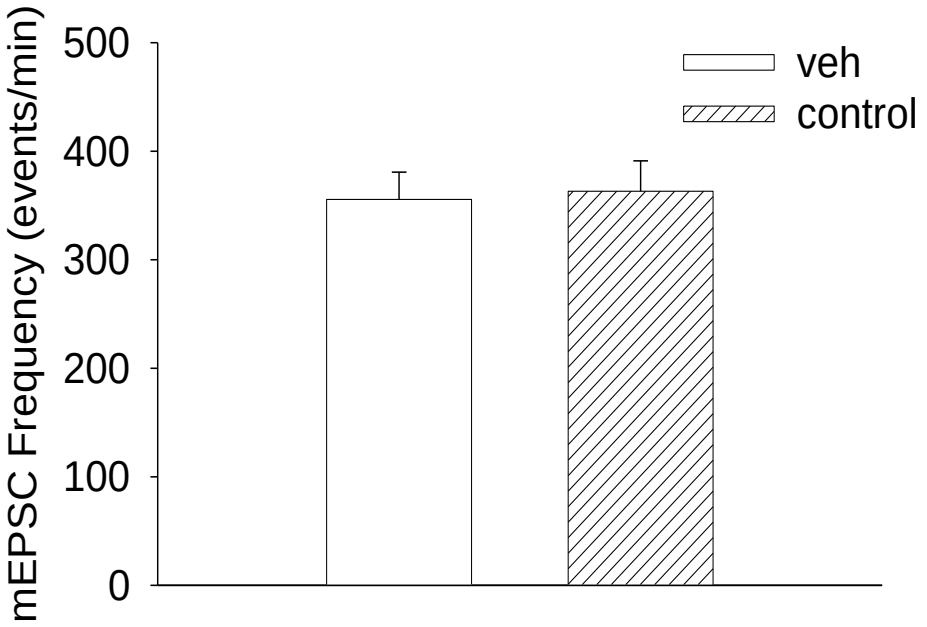
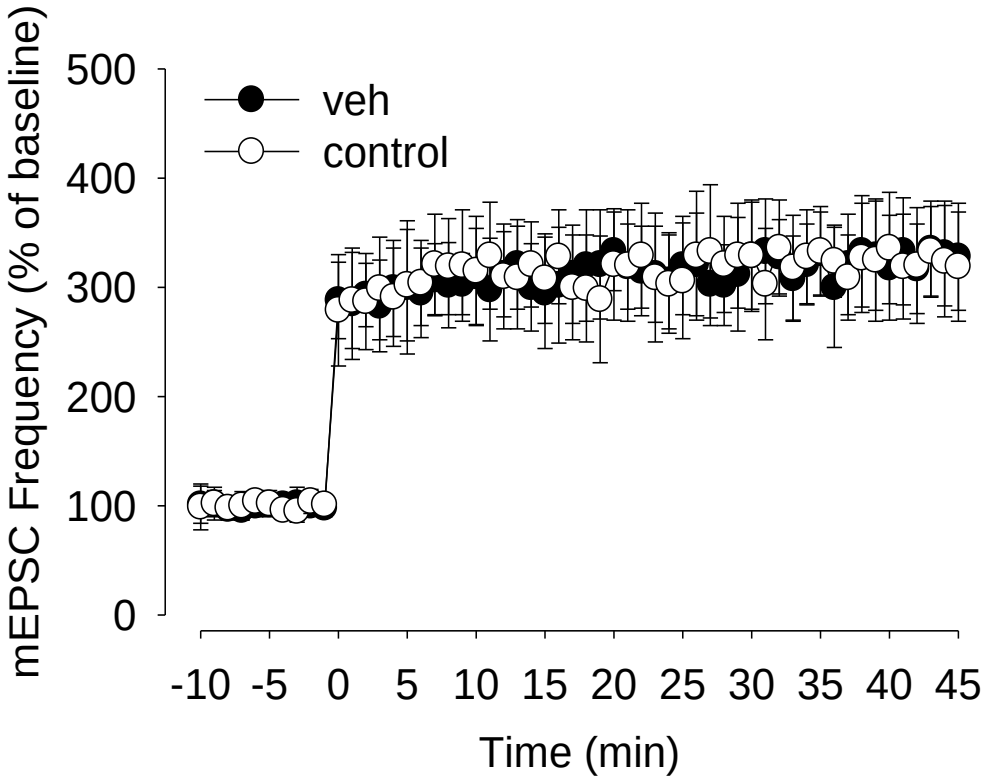
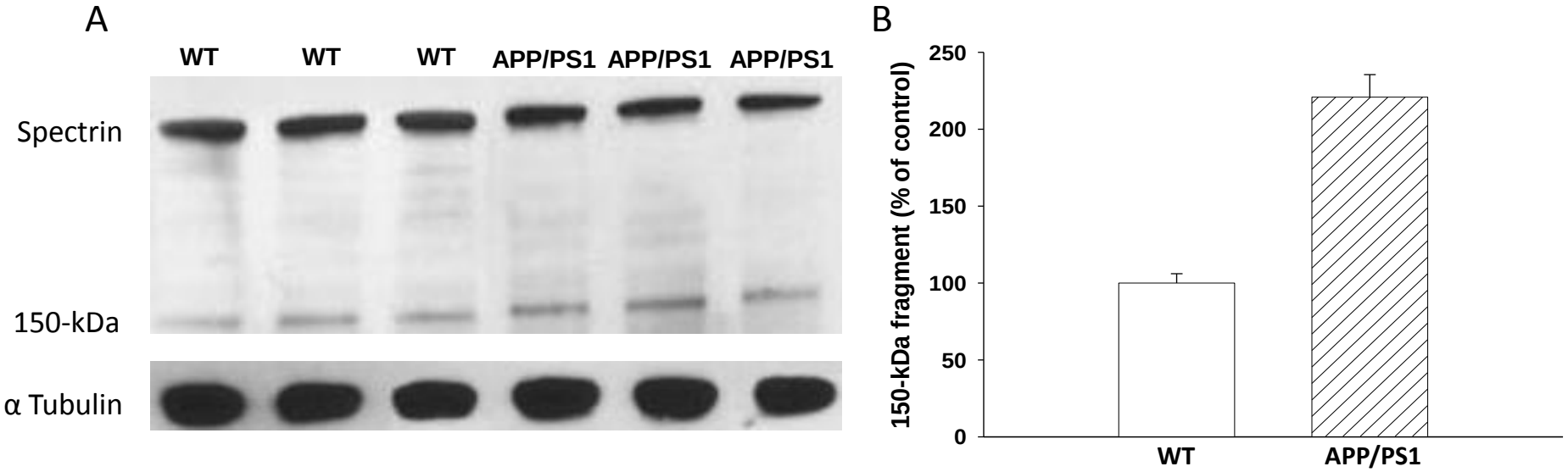
A

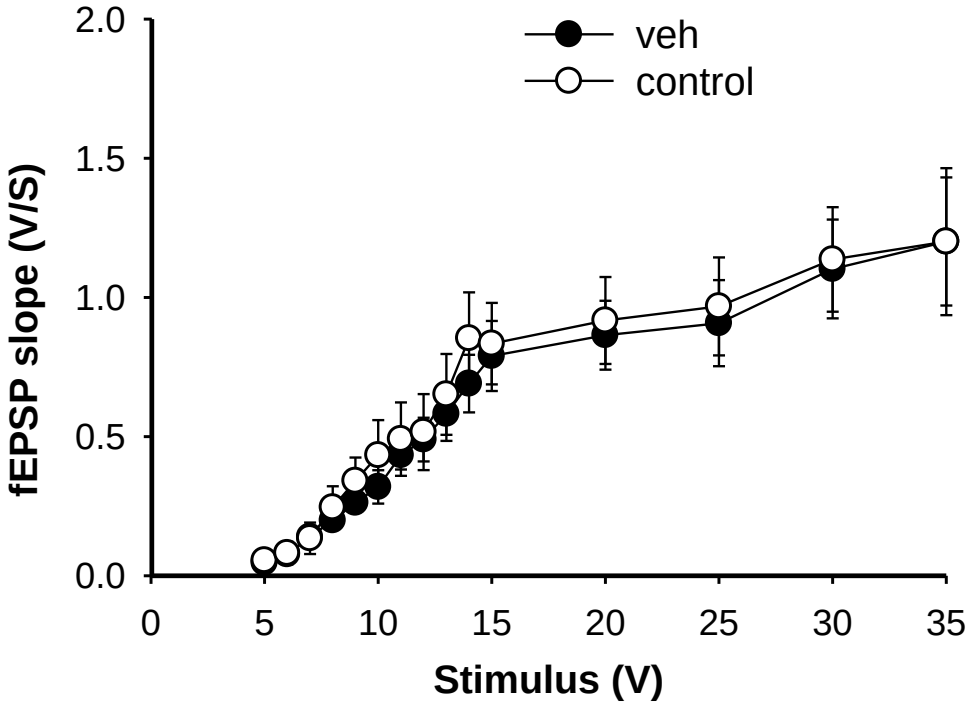

mEPSC Frequency (events/min)
500
400
300
200
100
0
veh
control

B

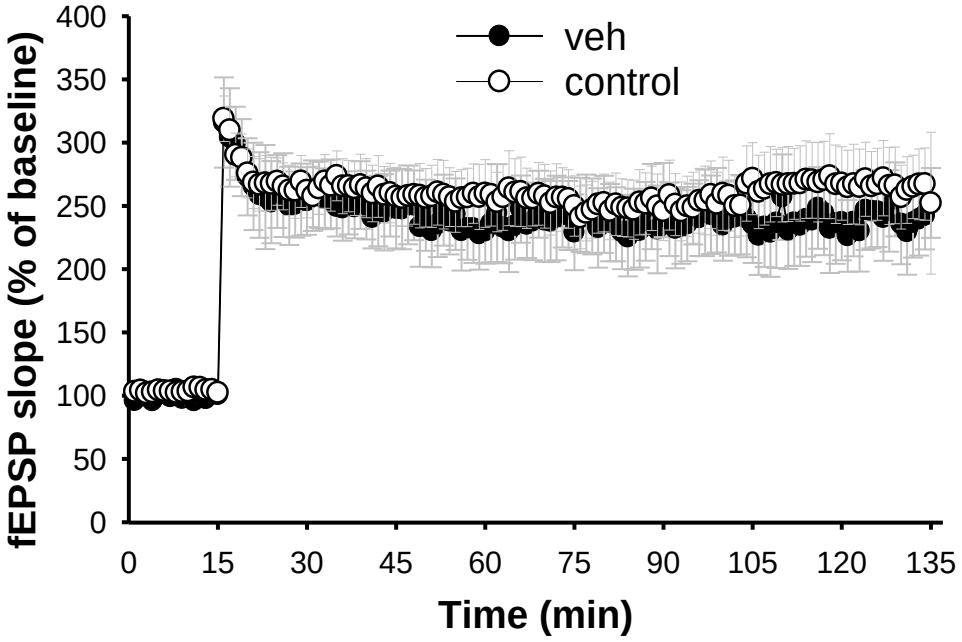

mEPSC Frequency (% of baseline)
500
400
300
200
100
0
veh
control
-10
-5
0
5
10
15
20
25
30
35
40
45
Time (min)


A
WT
WT
WT
APP/PS1
APP/PS1
APP/PS1
Spectrin
150-kDa
α Tubulin
B
150-kDa fragment (% of control)
250
200
150
100
50
0
WT
APP/PS1

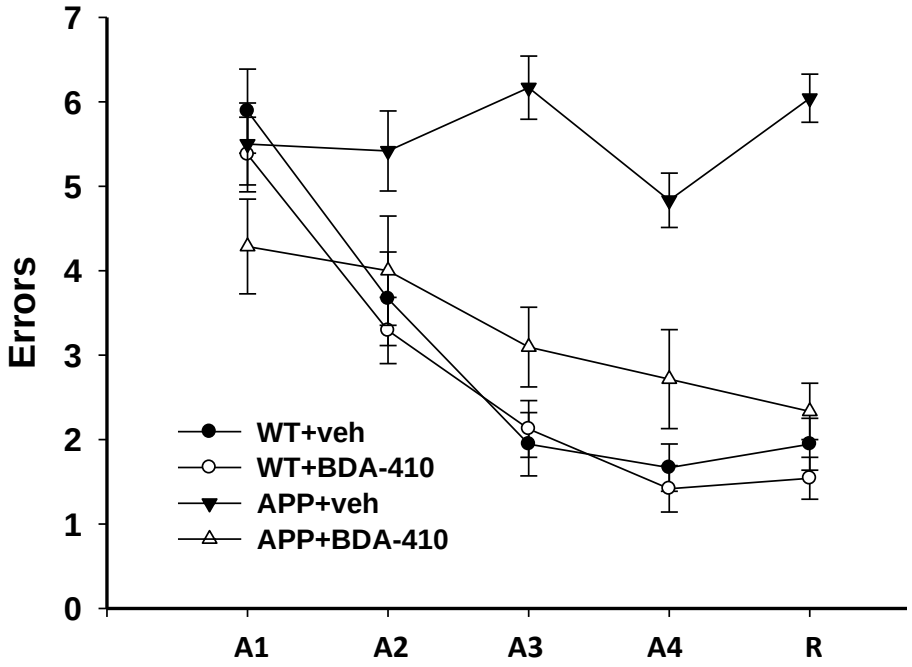
A


veh
control
fEPSP slope (V/S)
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
Stimulus (V)

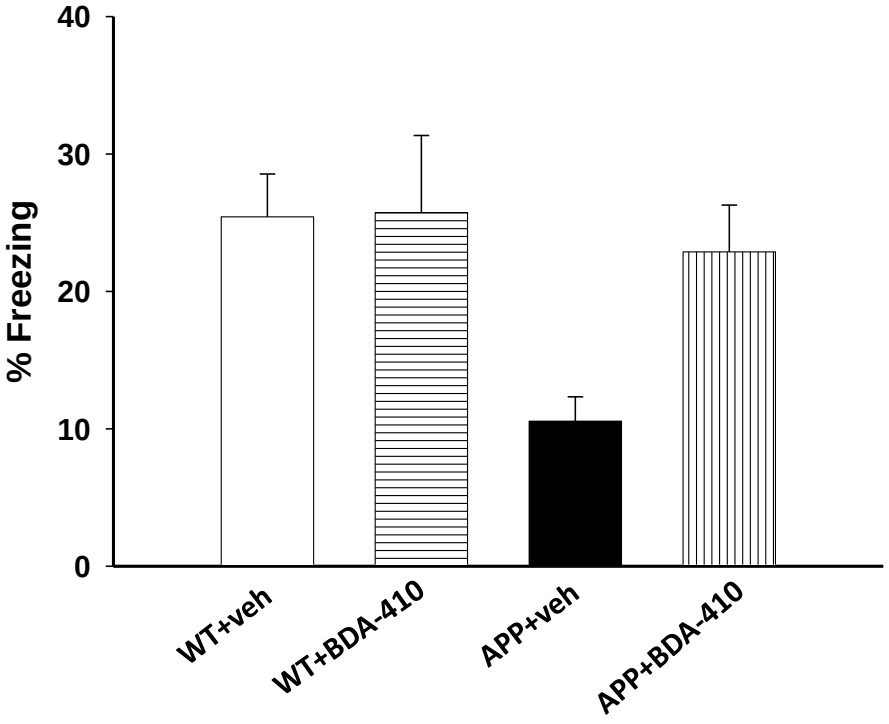
B

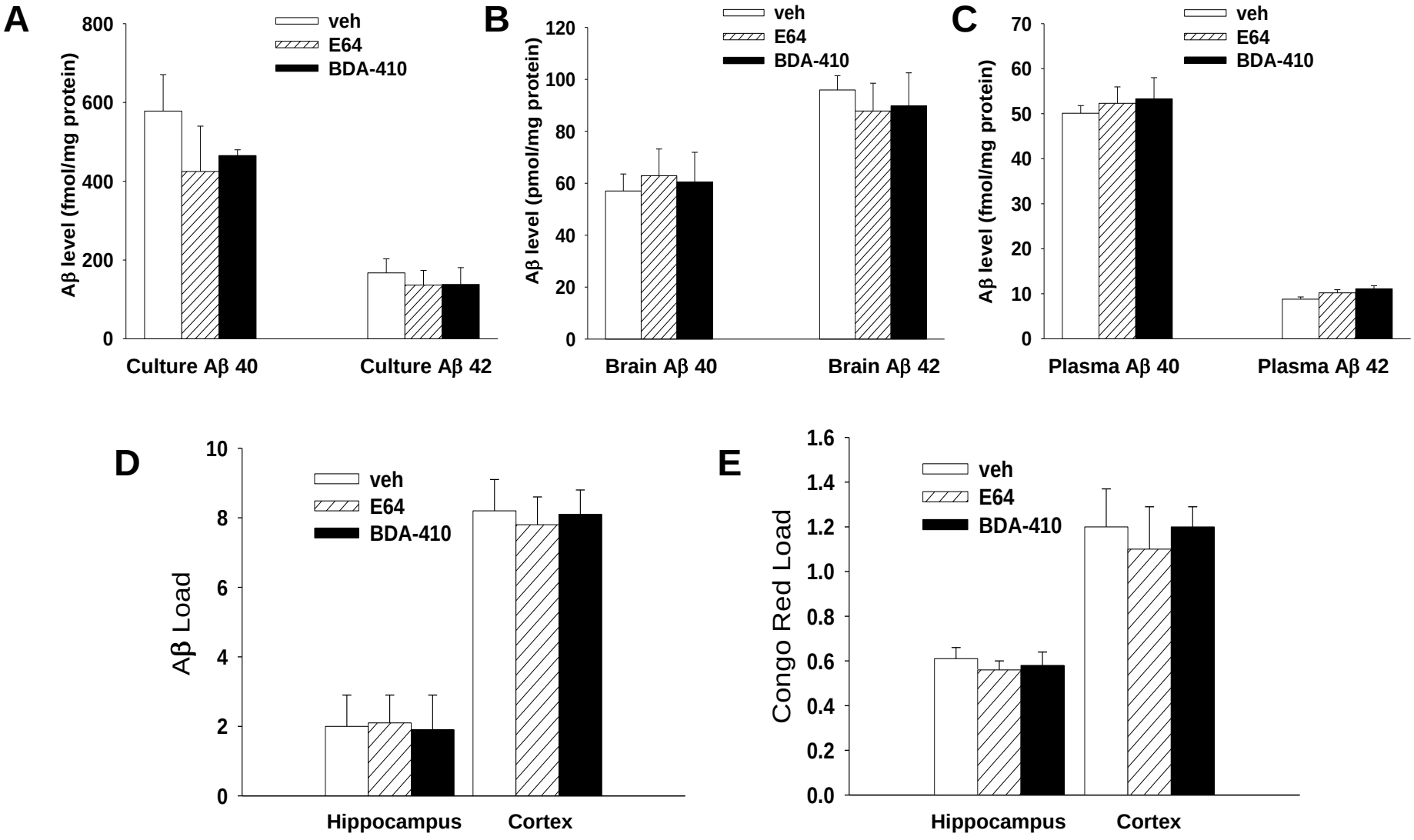

veh
control
fEPSP slope (% of baseline)
400
350
300
250
200
150
100
50
0
0
15
30
45
60
75
90
105
120
135
Time (min)

A


Errors
7
6
5
4
3
2
1
0
A1
A2
A3
A4
R
WT+veh
WT+BDA-410
APP+veh
APP+BDA-410

B


% Freezing
40
30
20
10
0
WT+veh
WT+BDA-410
APP+veh
APP+BDA-410


A
Aβ level (fmol/mg protein)
800
600
400
200
0
veh
E64
BDA-410
Culture Aβ 40
Culture Aβ 42
B
Aβ level (pmol/mg protein)
120
100
80
60
40
20
0
veh
E64
BDA-410
Brain Aβ 40
Brain Aβ 42
C
Aβ level (fmol/mg protein)
70
60
50
40
30
20
10
0
veh
E64
BDA-410
Plasma Aβ 40
Plasma Aβ 42
D
Aβ Load
10
8
6
4
2
0
veh
E64
BDA-410
Hippocampus
Cortex
E
Congo Red Load
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
veh
E64
BDA-410
Hippocampus
Cortex

SUPPLEMENTAL DATA

Supplemental methods

Immunocytochemistry on slices. Hippocampal slices were fixed in ice-cold 4% paraformaldehyde at 60 min after the treatment, washed three times in phosphate-buffered saline (PBS), treated with 0.3% Triton X-100 for 60 minutes, washed again for three times in PBS, treated with 50 mM ammonium chloride for 20 minutes and incubated in 10% goat serum for 60 minutes. Then, they were exposed to the primary antibody (rabbit polyclonal antiphospo-CREB from Upstate Biotechnology diluted 1:100 in 10% goat serum, for 36 hours at 4°C). Next, slices were washed in PBS (6 times, 2 hours each time), and treated with the secondary antibody (goat antirabbit antibody labeled with Alexa Fluor 488, from Molecular Probes, diluted 1:100 in 10% goat serum, for 12 hours at 4°C) and washed in PBS again (6 times, 2 hours each time). Slices were examined with a confocal microscope in blind. Kalman averages of 4 scans were collected for each image. Analysis was performed with NIH ImageJ software. The mean fluorescence intensity that exceeded a threshold set above background was determined for each slice in CA1 cell body area. The values were normalized to the values from untreated control slices from the same animal and expressed as mean percent of control ± SEM. For all the immunocytochemical experiments, the specificity of the immunofluorescence was confirmed by omitting the primary antibody, which resulted in a significant reduction in fluorescence intensity.

Behavioral Studies: The RAWM task has proven informative in the analysis of short-term memory of other transgenic AD models (1-4). Briefly, the RAWM consisted of a tank filled with opaque water by non toxic white paint. Walls were positioned so as to produce 6 arms, radiating from a central area. Spatial cues were present on the walls of the testing room. At the end of one of the arms there was a clear 10 cm submerged platform that remained in the same location for every trial on a given day, but was moved about randomly from day to day. Given that for each trial the mouse

started the task from a different randomly chosen arm, the mouse could not use its long-term memory of the location of the platform on previous days, but had to rely on the short-term memory of its location on the day in question based on spatial cues that were present in the room. Each trial lasted 1 min and errors were counted each time the mouse entered the wrong arm or needed more than 10 sec to reach the platform. After each error the mouse was pulled back to the start arm for that trial. After 4 consecutive acquisition trials, the mouse was placed in its home cage for 30 min, then returned to the maze and administered a 5^{th} retention trial. Testing was considered completed when the WT mice made the same number of errors during the 4^{th} and 5^{th} trial. The scores for each mouse on the last 3 days of testing were averaged and used for statistical analysis. Visible-platform training to test visual and motor deficits was performed in the same pool but without arms, with the platform marked with a black flag and positioned randomly from trial to trial. Each animal was allowed to swim for 1 min. Time to reach the platform and speed were recorded.

Fear conditioning is a form of learning impaired in several AD mouse models (4, 5). The test was performed as previously described (6). For fear conditioning we used a conditioning chamber located within a sound-attenuating box and containing a 36-bar insulated shock grid removable floor. For the cued and contextual conditioning experiments, mice were placed in the conditioning chamber for 2 min before the onset of a discrete tone (CS) (a sound that lasted 30 sec at 2800 Hz and 85 dB). In the last 2 sec of the CS, mice were given a foot shock (US) of 0.50 mA for 2 sec through the bars of the floor. After the CS/US pairing, mice were left in the conditioning chamber for another 30 sec and were then placed back in their home cages. Freezing behavior, defined as the absence of all movement except for that necessitated by breathing, were scored using the Freezeview software (MED Ass. Inc). To evaluate contextual fear learning, freezing was measured for 5 min in the chamber in which the mice were trained 24 hrs after training. To evaluate cued fear

learning, following contextual testing, mice were placed in a novel context (triangular cage with smooth flat floor and with vanilla odorant) for 2 min (pre-CS test), after which they were exposed to the CS for 3 min (CS test), and freezing was measured. Sensory perception of the shock was determined through threshold assessment, as described (4). When threshold to flinching, jumping, and screaming was quantified for each animal by averaging of the shock intensity at which each animal manifested a behavioral response of that type to the foot shock, we found a similar shock threshold among the various groups of mice (data not shown).

Supplemental Figures

Supplemental Figure 1. Treatment with vehicle did not affect synaptic function in WT cultures. **A** Vehicle-treated cultures showed similar basal mEPSC frequency (n = 5) as control WT cultures that did not receive any treatment (n = 5, $P > 0.05$). The average amplitude of mEPSCs in vehicle-treated WT cultures was similar as in control cultures (data not shown). **B** Vehicle-treated WT cultures showed a similar glutamate-induced increase in mEPSC frequency as control WT untreated cultures (n = 5 for both; $P > 0.05$ with a 2-way ANOVA). Glutamate application did not affect mEPSC amplitude in either groups (data not shown).

Supplemental Figure 2. Spectrin cleavage is increased in APP/PS1 mice. **A** Western blot for spectrin and its 150-kDa cleavage fragment in hippocampi of APP/PS1 and WT mice. **B** Quantitative Western blot analysis of data shown in A demonstrated a two-fold increase of the 150-kDa fragment levels in APP/PS1 mice compared to WT littermates (n = 3 for both, $P < 0.001$, data normalized against α-tubulin).

Supplemental Figure 3. Treatment with vehicle did not affect synaptic function in WT mice. **A** CA3-CA1 BST at hippocampal slices of 7-month-old vehicle-treated WT

mice was similar as that of control untreated mice (8 slices from 7 vehicle-treated WT mice, 6 slices from 6 control untreated mice, $P > 0.05$). **B** LTP at the same synapses as in **A** was not affected by the treatment with vehicle ($P > 0.05$).

Supplemental Figure 4. Calpain inhibition re-established normal spatial working memory and associative fear memory in APP mice. **A** BDA-410 re-established normal spatial working memory in 11-12 month old APP mice following 3 months of treatment. BDA-410 treated APP mice ($n = 7$) showed similar performance in the RAWM task as vehicle-treated WT littermates ($n = 6$), whereas vehicle-treated APP mice ($n = 8$) showed abnormal learning and memory [$P < 0.05$ in BDA-410 treated APP mice compared to vehicle treated APP mice, planned comparisons showed that the 2 groups were significantly different at trial A3, A4 and R ($P < 0.01$)]. The inhibitor did not affect the performance of WT mice ($n = 8$; $P > 0.05$ compared to vehicle-treated WT mice). The visible platform task did not show any sensory-motor impairment in all groups (data not shown). **B** BDA-410 re-established normal contextual learning in 11-12 month old APP mice following 3 months of treatment ($n = 12$; $P < 0.01$ compared to vehicle-treated APP mice showing a decreased freezing behavior, $n = 11$ vs $n = 8$ in WT littermates). The inhibitor did not affect the performance of WT mice ($n = 7$, $P > 0.05$). Cued learning did not show any difference among the four groups (data not shown).

Supplemental Figure 5. Calpain inhibition did not affect A_ levels in APP/PS1 mice. **A** $A\beta_{40}$ and $A\beta_{42}$ concentrations measured through enzyme-linked immunosorbent assay system (ELISA) in the medium of APP/PS1 cultured neurons. 3-4 days treatment with E64 and BDA-410 did not affect the levels of $A\beta_{40}$ and $A\beta_{42}$ ($n = 7$ for all the experiments in which dishes were treated with E64 and n = 6 for all the experiments in which dishes were treated with BDA-410; $P > 0.05$ compared to

vehicle-treated dishes from APP/PS1 mice for both $A\beta_{40}$ and $A\beta_{42}$). **B and C** E64 and BDA-410 did not affect formic acid extractable brain **(B)** and plasma $A\beta$ levels **(C)** in APP/PS1 mice. Transgenic mice were injected from the age of 8 weeks for 5 months. No difference was observed between E64- and BDA-410-injected mice compared to vehicle-injected animals (*n* = 5 for all the experiments in which animals were treated with E64 and *n* = 6 for all the experiments in which mice were treated with BDA-410). **D and E** E64 and BDA-410 did not affect plaque load in APP/PS1 mice. Plaques were stained with 4G8 antibodies (D) and Congo red (E) at the level of hippocampus and cortex. Transgenic mice were injected from the age of 8 weeks for 5 months (*n* = 5 for all the experiments in which animals were treated with E64 and *n* = 6 for all the experiments in which mice were treated with BDA-410).

Supplemental References

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