Amyloid β-Protein Dimers Isolated Directly from Alzheimer Brains Impair Synaptic Plasticity and Memory

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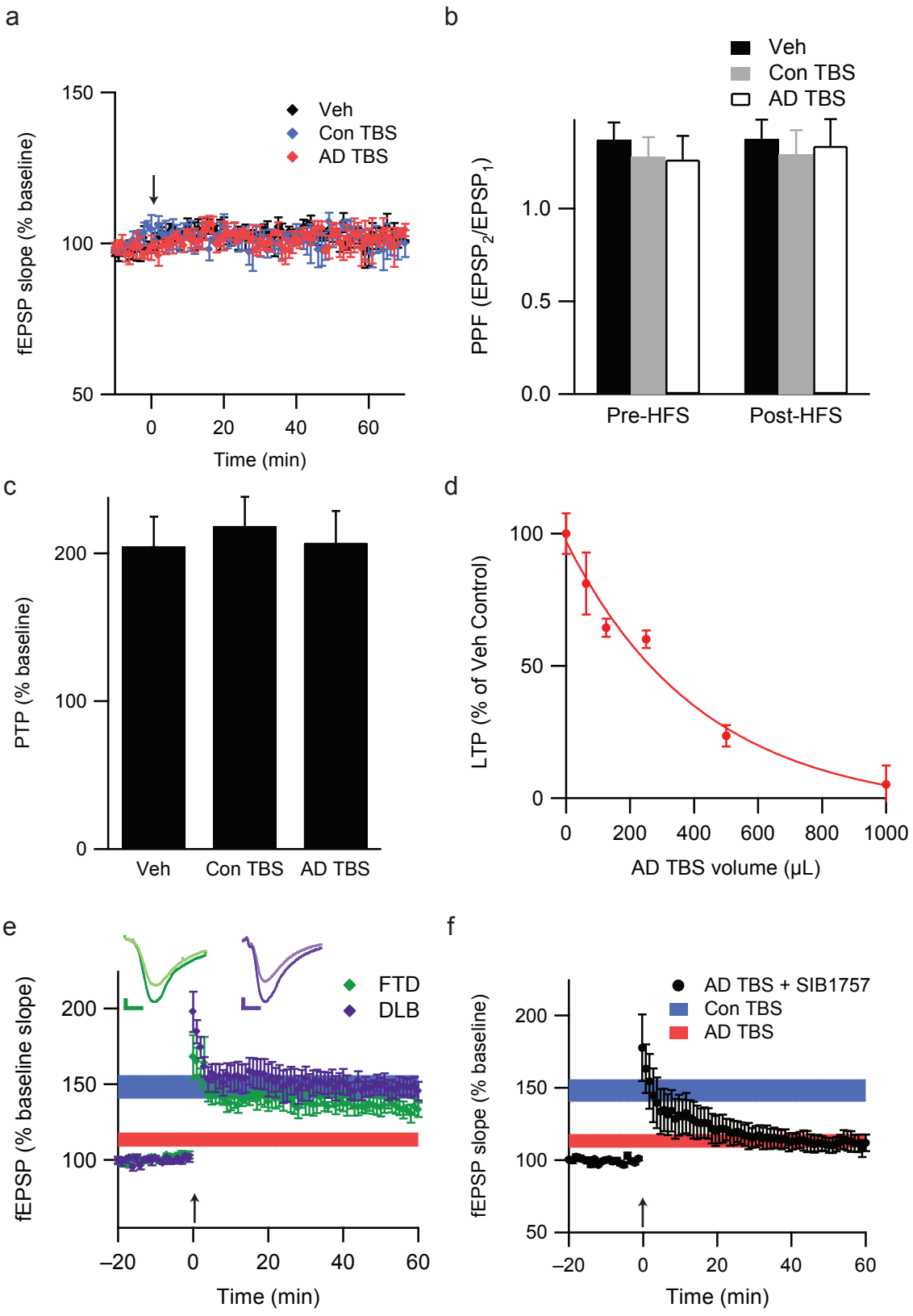
SUPPLEMENTAL FIGURE LEGENDS

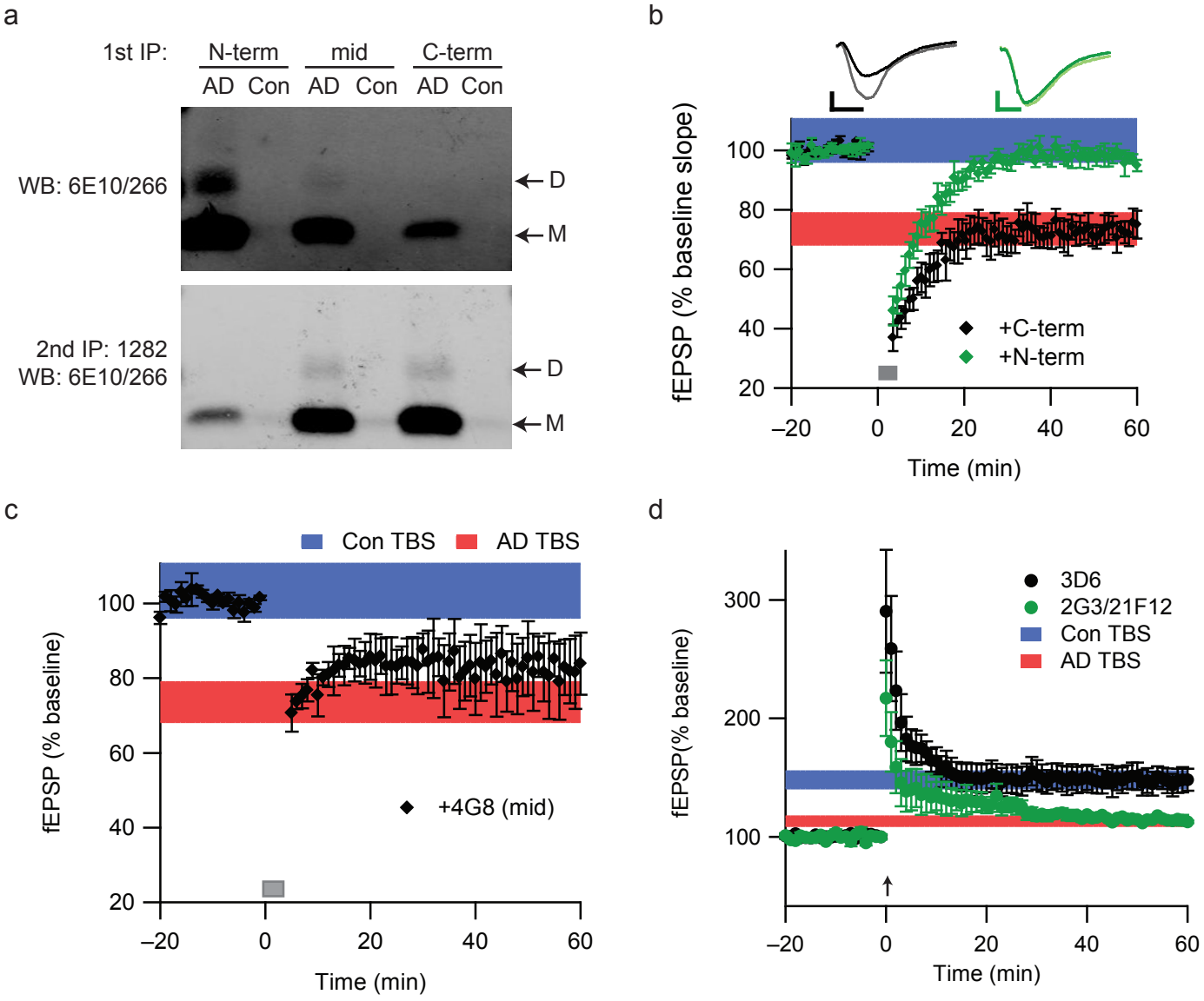
Supplemental Figure 1. AD soluble (TBS) brain extract does not affect baseline synaptic transmission or presynaptic release probability, but causes dose dependent LTP inhibition. **a**. After recording fEPSPs for 10 min, 500 μL TBS Veh, Con TBS extract or AD TBS extract were added to the ACSF bath (time indicated by arrow). fEPSPs were recorded for an additional 70 min following this wash-in. **b**. Paired pulse facilitation (PPF) was calculated from the slope of the two fEPSPs resulting from an inter-stimulus interval of 50 msec. ANOVA was performed on average PPF 5 min prior to HFS (Pre-HFS) or for 55–60 min after HFS (post-HFS) from slices exposed to TBS Veh, Con TBS or AD TBS. **c**. Post-tetanic potentiation (PTP) calculated from fEPSPs recorded 5 min following HFS. **d**. The LTP inhibition by Aβ in the soluble (TBS) extract of AD cortex is dose dependent. Data are plotted as percentages of optimal LTP (i.e., that obtained in 500 μL plain TBS) measured at 50–60 min following HFS. Each dose is indicated on the abscissa as the volume of AD TBS extract that was added to a volume of plain TBS to yield a final sample volume of 500 μL. In the case of the 500 μL and 1000 μL samples, the AD TBS extract was added straight. Dashed line indicates approximation of IC_{50} value for AD TBS, which corresponds to a final Aβ concentration of ~7 pM in the slice perfusate. **e**. TBS cortical extracts from humans with confirmed frontotemporal dementia (FTD, Fig. 1) or dementia with Lewy bodies (DLB, Fig. 1) do not inhibit LTP induction. Summary data for LTP induction in slices treated with 500 μL TBS extracts of brains with either FTD (green) or DLB (purple). For comparison, LTP data from Fig. 2b (means ± SEM) for Con TBS (blue) and AD TBS (red) are shown as horizontal bars. **f**. The mGluR5 anatagonist SIB1757 does not block the effect of AD TBS on LTP. Summary LTP data for co-administration of 3 uM SIB1757 with 500 μL AD TBS (n=5 slices) for 20 minutes prior to the HFS (indicated by arrow). For comparison, LTP data from Fig. 2b (means ± SEM) for Con TBS (blue) and AD TBS (red) are shown as horizontal bars.

Supplemental Figure 1



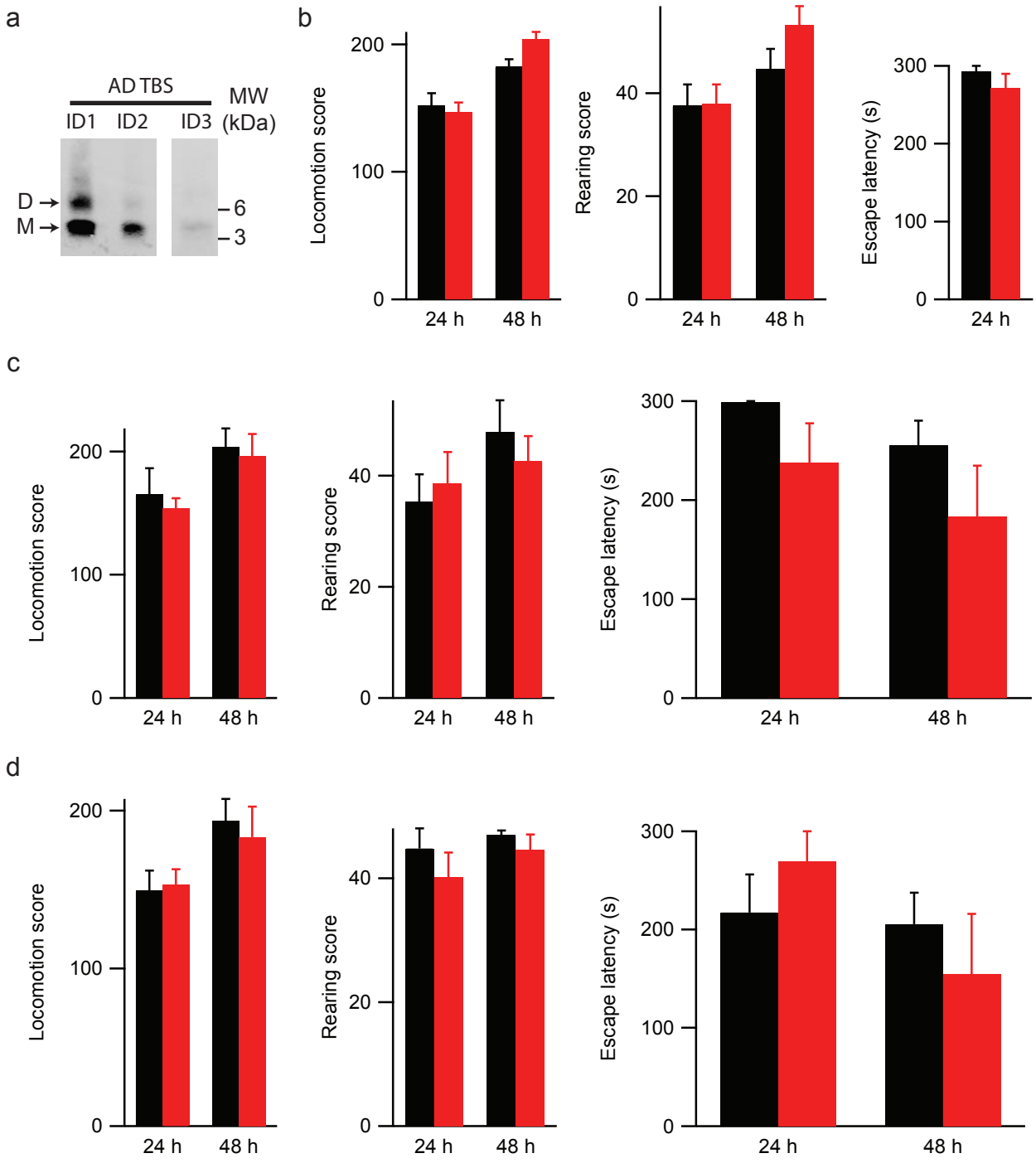
Supplemental Figure 2. N-terminal antibodies recognize Aβ dimers and neutralize their effects on synaptic plasticity more effectively than antibodies targeting the Aβ mid-region. **a**. AD TBS and Con TBS was first IP'd with 3 μg/mL of either 3D6 (N-term), 4G8 (mid) or 2G3/21F12 (C-term) Aβ antibodies (see text) and probed with Aβ antibodies 6E10 plus 266. The sup after this first IP was then IP'ed with polyclonal Aβ antibody R1282 (2^{nd} IP) and probed with 6E10 plus 266 to detect remaining Aβ species. **b**. Summary LTD data for co-administration of AD TBS with 3 μg/mL of N-terminal (+N-term, green) or C-terminal (+C-term, black) Aβ antibodies. LTD data (means ± SEM) for Con TBS (blue) and AD TBS (red) are shown as horizontal bars. **c**. Summary LTD data (means ± SEM) for co-administration of AD TBS with 3 μg/mL 4G8 (+4G8, black, n=3 slices) for 20 min prior to LFS (grey bar). LTD data for Con TBS (blue) and AD TBS (red) from Fig. 2e are shown as horizontal bars. **d**. Summary LTP data (means ± SEM) for co-administration of AD TBS with 3 μg/mL 3D6 (N-term, black, n=5 slices) or 2G3/21F12 (C-term, green, n=5 slices) for 20 min prior to HFS (arrow). LTD data for Con TBS (blue) and AD TBS (red) are shown as horizontal bars.

Supplemental Figure 2



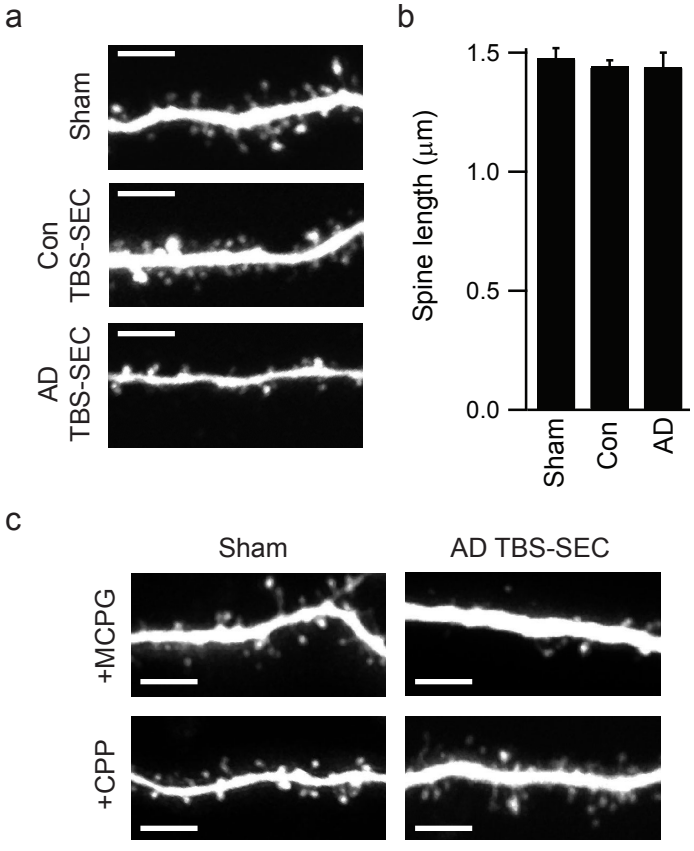
Supplemental Figure 3. Introduction of AD TBS at either 0 h or 6 h following training in a passive avoidance paradigm does not significantly impair the recall of this learned behavior. **a**. WB of three sequential IP's of the TBS extract (AD TBS). AD TBS-ID is the TBS supernatant remaining after the third immunodepletion (ID3). AD TBS (red) or AD TBS-ID (immunodepleted, black) was introduced i.c.v. into the lateral ventricle at either (**b**) 3 h, or (**c**) 0 h, or (**d**) 6 h following training. No significant differences between the two treatment groups were observed in locomotion scores (left) or rearing scores (middle) measured at 48 or 24 h prior to training. Furthermore, the escape latency (right) was not significantly different at 24 h or 48 h post-training when comparing rats receiving AD TBS (n=5 and 5 rats for injections at 0 h and 6 h) or AD TBS-ID (also n=5 and 5). While the escape latency was not significantly different at 24 h post-training between rats treated at 3 h with AD TBS or AD TBS-ID (b, right), a significant difference was observed at 48 h (see Fig. 2h).

Supplemental Figure 3



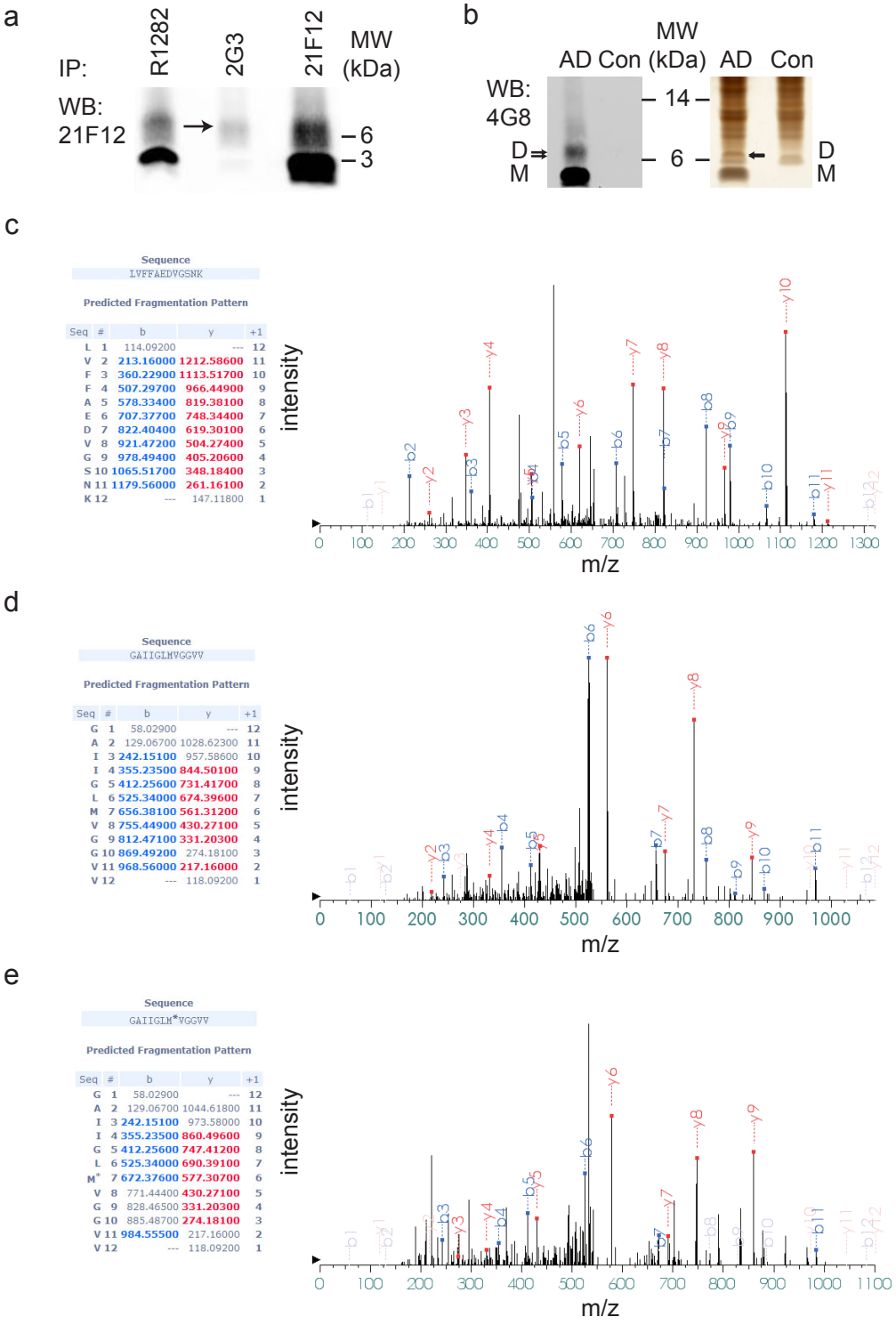
Supplemental Figure 4. Soluble Aβ from AD brain decreases dendritic spine density through a NMDAR-dependent pathway. **a.** Representative images of apical dendrites from 5 DIV pyramidal cells in organotypic hippocampal slices cultured for an additional 10 days in sham condition or with SEC-enriched TBS extract from AD brain (AD TBS-SEC) or from Con brain (Con TBS-SEC). Scale bar = 5 μm. **b.** Summary data for spine length. **c.** Representative images of slices treated with 500 μM MCPG or 20 μM CPP in the absence (sham) or presence of AD TBS-SEC.

Supplemental Figure 4



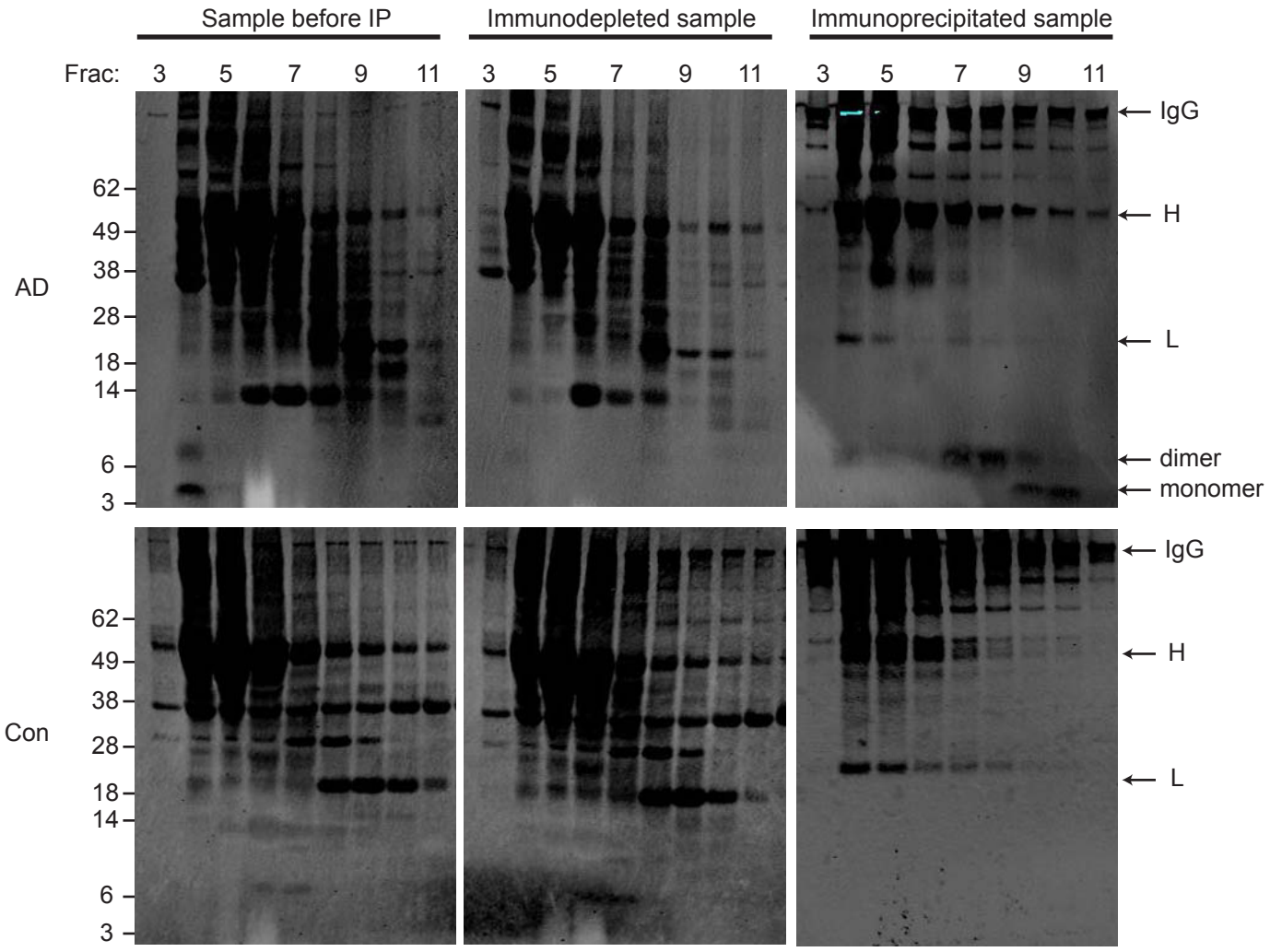
Supplemental Figure 5. Mass spectrometric confirmation of human Aβ in the 8 kDa dimer band after IP of the GuHCl extract of an AD brain. **a.** Insoluble (GuHCl) extract of AD brain was IP'ed with either 2G3 or 21F12 (or R1282 as a control), and all 3 precipitates were probed with 21F12. 21F12 detects an $A\beta_{40/42}$ heterodimer (arrow) in the 2G3 IP but detects far more Aβ dimers in the 21F12 IP, representing $A\beta_{42/42}$ homodimers. **b.** GuHCl extracts of AD or control brain were IP'ed with a combination of 266, 2G3 and 21F12. WB with 4G8 (left panel) and silver staining (right panel) reveal bands in AD but not Con brain that correspond to the sizes of Aβ monomer (M) and dimer (D). Bands identified as Aβ monomer and dimer by WB and silver stain were digested with trypsin and the resulting peptides analyzed by LC-MS/MS. Analysis of the dimer band provided the sequences of: **c**. residues 17–28; **d**. residues 29–40; and **e**. residues 29–40 with an oxidized methionine at position 35 (a modification previously described in Aβ from AD cortex[1]). The predicted peptide fragments are displayed vertically on the left and the observed mass-to-charge ratios are displayed horizontally on the right. Analysis of the monomer band provided the same peptide sequences (not shown). We did not recover the N-terminal tryptic fragment of Aβ (residues 1–16) from either the 4 or 8 kDa bands, but IP with Asp-1 specific N-terminal Aβ antibodies confirmed that these bands both contain Aβ beginning at Asp-1 (e.g., see Supp. Fig. 2a).

Supplemental Figure 5



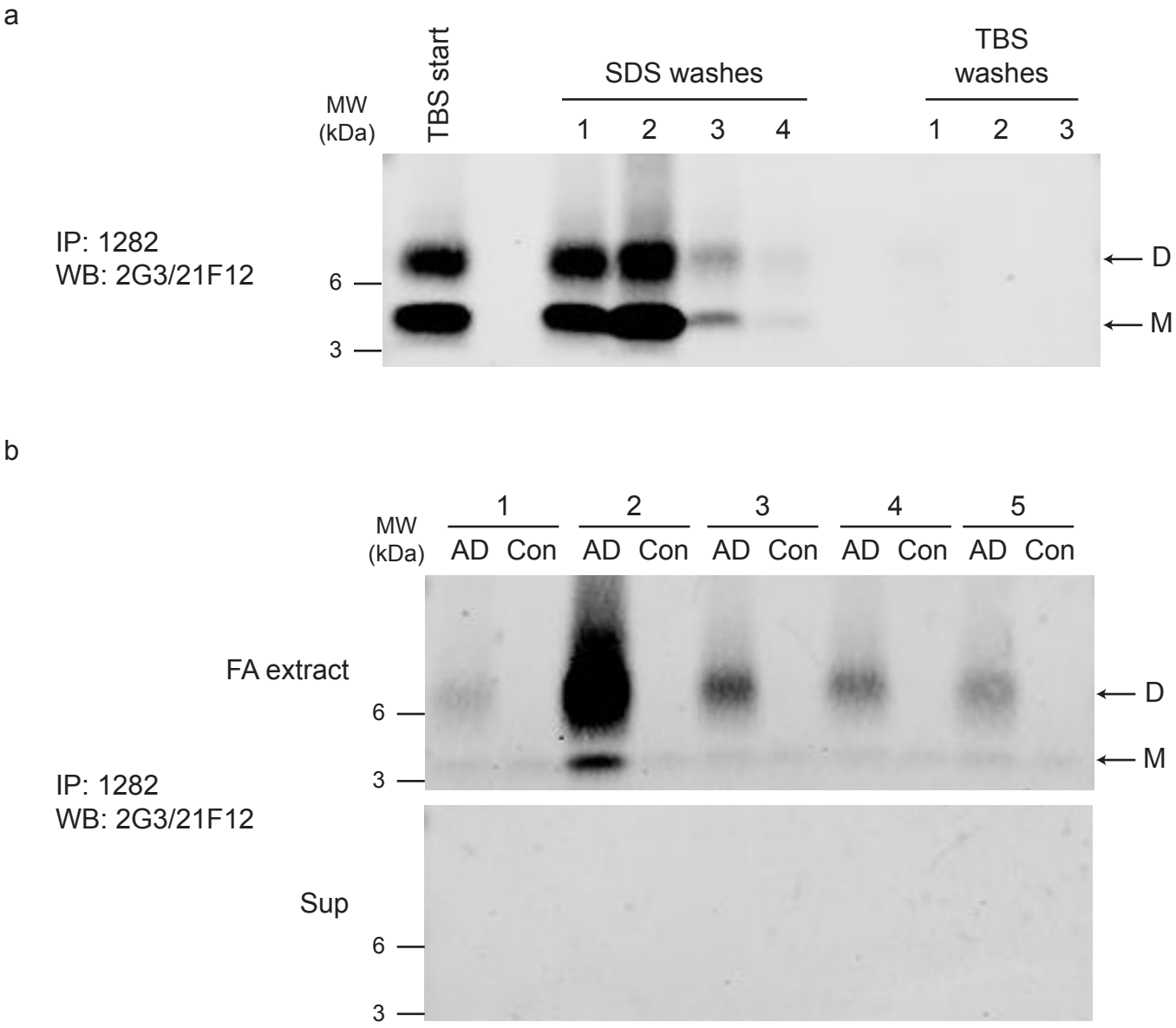
Supplemental Figure 6. Aβ in AD TBS extracts can be purified and resolved as oligomer- and monomer-rich fractions by IP followed by SEC. AD TBS (top) or Con TBS (bottom) was IP'ed with 3D6 (3 μg/mL), eluted with sample buffer containing 4% LDS and subjected to SEC. SEC of AD TBS without IP (top left) reveals that Aβ monomers and dimers elute in fraction 4, which corresponds to the column void volume. This is detected by Aβ C-terminal antibodies 2G3/21F12. Immunodepleted AD TBS (following IP with 3D6) reveals a similar pattern of background bands detected by WB (top center). SEC of the IP (top right) resolves Aβ dimers (fractions 7/8) from monomers (fractions 9/10), as detected by 2G3/21F12 and 4G8. Fractions from the IP-SEC sample also contain intact immunoglobulin (IgG), and heavy chain (H) and light chain (L) fragments contributed by the immunoprecipitating antibody, as detected under these non-reducing conditions. As seen here and also by silver stain (not shown), the Aβ-containing fractions following IP-SEC (top right) contain less background protein compared to the starting AD TBS extract (top left). Comparison of AD TBS and Con TBS blots reveals that the only novel species are the 4 and 8 kDa bands that exist only in AD TBS (not Con TBS, lower panels) and represent Aβ monomer and dimer, respectively.

Supplemental Figure 6



Supplemental Figure 7. Amyloid cores enriched from human AD cortex consist of dimers that are not readily released under physiologic conditions. **a.** Amyloid cores were prepared from AD brain tissue (100 mg wet wt) through sequential extractions with TBS and 2% SDS (as described in Methods and Figs. 6a, b). IP/WB analyses of wash supernatants reveal no additional soluble Aβ species are liberated from the core prep by three final TBS washes after 4 sequential SDS washes. **b.** Amyloid cores remaining after the 7 sequential washes in (a) were subjected to a variety of incubation conditions to attempt the release of soluble Aβ species. Sample 1 was cores prepared from 100 mg brain wet wt as in (a) and probed without further incubation. Samples 2–5 were cores prepared from 100 mg (samples 3–5) or 500 mg (sample 2) and then all incubated at 37°C for 24 hr. Samples 2 and 3 were incubated in 1% BSA in TBS (BSA was used to prevent non-specific loss of Aβ on the wall of the siliconized tube during incubation). Sample 4 was sonicated for 1 min to increase amyloid core surface area then incubated in 1% BSA in TBS. Sample 5 was incubated in Con TBS to determine whether any soluble proteins in human brain extract could liberate Aβ from the cores. IP/WB analysis of the post-500 g supernatants (bottom panel) and formic acid (FA) extracted pellets (top panel) reveal Aβ dimers in the FA extracts of the remaining core pellet, but no soluble Aβ released into the supernatant by these various treatments.

Supplemental Figure 7



Supplemental Table 1. a. Clinical and histopathological information on brain samples used for analysis in Fig. 1a. **b.** Clinical and histopathological information on brain samples used for analysis in Fig. 2a. Total Aβ levels measured by ELISA in a subset of cases were 1.93 to 2.34 nmol Aβ/g brain tissue in the insoluble extracts and 0.04 to 1.44 pmol Aβ/g brain tissue in the soluble extracts of AD cortex, in range with previous reports[2,3].

a

Case ID	Age	Clinical presentation	Neuropathological diagnosis
DS	60	Down syndrome, AD	AD
DLB	74	Possible CJD	Dementia with Lewy bodies
PCD	64	Recent onset of confusion and agitation	Paraneoplastic cerebellar degeneration
AD 1	73	Dementia	AD
MID 1	75	Recent onset of confusion	Stroke
AD 2	68	Dementia	AD
P-AD	78	Normal pressure hydrocephalus	AD
FTD	50	Frontotemporal dementia	Frontotemporal dementia
MID 2	69	Dementia	Multi-infarct dementia
AD 3	85	Dementia	AD

b

Case ID	Age	Clinical presentation	Neuropathological diagnosis
Con 1	75	Neurologically normal	Colloid cyst of third ventricle
Con 2	70	atypical dementia, possible AD or FTD	FTD
Con 3	52	Neurologically normal	Ventriculomegaly
AD 4	81	AD	AD
AD 5	80	AD	AD
AD 6	78	AD	AD
AD 7	82	AD	AD

SUPPLEMENTARY METHODS

Hippocampal slice preparation for electrophysiology recording. Mice (C57BL/6 x 129, 5-8 wk old) were sacrificed after anesthesia with Isofen. The brain was quickly removed and submerged in ice-cold, oxygenated, sucrose-replaced artificial cerebrospinal fluid (ACSF) cutting solution (all in mM): 206 sucrose, 2 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 1 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 10 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 (all in mM): 124 NaCl, 2 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose, pH 7.4, 310 mOsm, in which buffer 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 a continuous ACSF perfusate saturated with 95% O_2 and 5% CO_2. Slices were incubated in the recording chamber for 20 min before stimulation at RT (~26°C).

Dendritic spine density analysis. The apical dendrites of pyramidal cells in organotypic hippocampal slices were prepared, treated, imaged and analyzed as described[4]. Briefly, 2 days after organotypic hippocampal slices were prepared from postnatal day 5 (P5) - P7 Sprague-Dawley rats, the slices were biolistically transfected with eGFP-N1 (Clontech, Cambridge, UK). After an additional 3 d of culturing, the various reported treatments were performed for an additional 10 d. All Aβ containing SEC fractions from AD TBS (AD TBS-SEC) and the corresponding fractions from Con TBS (Con TBS-SEC) were pooled separately and lyophilized prior to addition to the culture medium of organotypic hippocampal slice preparations. For treatment with Con TBS-SEC or AD TBS-SEC 350 μL lyophilized SEC fractions of the human brain TBS extract were reconstituted in 3 mL slice culture medium (SCM). 750 μL of this prep were applied to each insert in a 6-well plate. Either D-CPP (20 μM) or (R/S)-MCPG (500 μM) were added to sham or AD TBS-gf treated slices. For treatment with the GuHCl extract SEC fractions, a 450 μL lyophilized fraction was reconstituted in 4.5 mL SCM, of which 750 μL were added to each insert. The media were changed with fresh medium containing these treatments every 2-3 d. At the end of the 10-day treatment, live-cell imaging was performed on the inserts after submerging in ACSF. Apical dendrites were imaged by 2-photon laser scanning microscopy using a 5x zoom corresponding to 42 μm x 42 μm. Analysis on the acquired images was performed blinded to treatment using custom software designed in Matlab. Statistical analyses were performed using Igor Pro (Wavemetrics, Lake Oswego, OR).

Passive Avoidance Conditioning. Passive avoidance training was performed as described previously[5] (see Supplemental Methods). Wistar rats were obtained from the Biomedical Facility at University College Dublin, and all procedures were conducted in accordance with animal welfare guidelines of the Department of Health and Children (Republic of Ireland). Animals were cannulated at P70 and handled for 6 days prior to training at P80. Weight was measured and behavior was assessed in an open-field apparatus for 5 min periods at 48 and 24 h before training. Locomotion, rearing, grooming, piloerection, defecation and posture were studied to assess post-surgical alterations in behavior. Animals were trained in a single-trial, step-through, light–dark passive avoidance paradigm. The training apparatus chamber was divided into two

compartments, separated by a central shutter that contained a small opening. The smaller of the compartments contained a low-power illumination source; the larger compartment was not illuminated. The floor in the dark compartment consisted of a grid of 16 horizontal stainless-steel bars. A current generator supplied 0.75 mA to the floor, which was scrambled once every 0.5s across the 16 bars. A resistance range of 40–60 mΩ was calculated for a control group of rats (250–350 g), and the apparatus was calibrated accordingly. On the day of training, animals were placed facing the rear of the light compartment of the apparatus, immediately after spontaneous behavior was assessed. The timer was started once the animal completely turned to face the front of the chamber. Latency to enter the dark chamber was recorded (usually <20 s). After the animal completely entered the dark compartment a foot shock was administered to the animal, at which point the subject immediately returned to the light compartment. Animals were then returned to their home cages. At 0, 3, or 6h following this training session, 5 μL soluble human cortical extract was introduced i.c.v. through the cannula at 1μL/min. Recall of the passive avoidance conditioning was evaluated 24 and 48 h post-training by recording the latency to enter the dark chamber, with a criterion time of 300s. Data from the passive avoidance studies were analyzed by ANOVA followed by the Bonferroni post hoc test.

IP-SEC. TBS extracts of AD or control cortex were immunoprecipitated with 3D6 (3 μg/mL), 15 μL PAS and 15 μL PGA. After the beads were washed, the immunoprecipitates were eluted with 10 μL 4% LDS sample buffer, heated at 65°C for 5 min and centrifuged at 14,000 rpm for 5 min. The supernate was transferred to 500 μL TBS and subjected to SEC as described above.

Quantification of Aβ in brain extract. Aβ levels in the brain extract were analyzed by sandwich enzyme-linked immunosorbent assay (ELISA) as described previously [6]. Briefly, 2G3 and 21F12 was used to capture $A\beta_{40}$ and $A\beta_{42}$, respectively. Following capture, biotinylated 3D6 or biotinylated 4G8 was used to quantify TBS or GuHCl extracted Aβ, respectively.

Mass spec methods. Mass spectrometry experiments were performed at the Taplin Biological Mass Spectrometry Facility at Harvard Medical School. Gel bands containing Aβ samples were digested with trypsin and then analyzed by nanoscale microcapillary liquid chromatography coupled to tandem mass spectrometry as described [7].

Production & characterization of cross-linked synthetic dimers. $A\beta_{1\text{-}40}$S26C was synthesized by the Biopolymer Laboratory at UCLA Medical School and the correct sequence and purity confirmed by amino acid analysis, reverse-phase HPLC and mass spectrometry. Aβ dimers were generated by atmospheric oxidation of a 20 μM solution of $A\beta_{1\text{-}40}$S26C in 20 mM ammonium bicarbonate, pH 8.0, for 4 days at room temperature. To facilitate disassembly of aggregates formed during the oxidation reaction, the peptide solution was lyophilized and subsequently incubated in 5 M GuHCl, Tris-HCl, pH 8.0, for 4 hr. Disulfide crossed-linked Aβ dimers were isolated from unreacted monomer and higher aggregates by size exclusion chromatography. Briefly, two Superdex 75 10/30 HR columns were linked in series and eluted with 50 mM

ammonium acetate, pH 8.5 at a flow rate of 0.5 ml/min. Fractions (0.5 ml) were collected, and an aliquot of each was electrophoresed on 16% tris-tricine polyacrylamide gels and detected by silver staining. Fractions found to contain exclusively dimeric Aβ were pooled and the peptide content determined by comparison to known standards. Samples were stored at -80^{o}C until used.

Isolation of amyloid cores. The pellet resulting from spinning the TBS homogenate at 175,000 g for 30 min (TBS start) was resuspended in 150 μL 2% SDS per 100 mg starting brain wet weight, boiled at 100°C for 5 min, and centrifuged at 10,000g for 5 min. The supernatant was collected (SDS Wash 1) and the pellet was extracted similarly with SDS an additional 3 times (SDS Washes 2-4). The remaining pellet was resuspended in 150 μL TBS per 100 mg starting brain wet weight and centrifuged at 500 g for 5 min. The supernatant was collected (TBS Wash 1) and the pellet was washed with TBS an additional two times (TBS Wash 2-3). The resulting washed core prep (following a final centrifugation at 500 g for 5 min) was incubated with 10 μL 88% formic acid per 100 mg starting brain weight at RT for 2 hr. This sample was neutralized with 2.6 vol 10 N NaOH prior to IP/WB analysis or treatment of acute hippocampal slices. Intact cores (following the final TBS wash) were centrifuged at 500 g and resuspended in 0.2% Congo red for visualization by brightfield and polarized microscopy.

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