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LETTERS

Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory

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Alzheimer's disease constitutes a rising threat to public health. Despite extensive research in cellular and animal models, identifying the pathogenic agent present in the human brain and showing that it confers key features of Alzheimer's disease has not been achieved. We extracted soluble amyloid-β protein (Aβ) oligomers directly from the cerebral cortex of subjects with Alzheimer's disease. The oligomers potently inhibited long-term potentiation (LTP), enhanced long-term depression (LTD) and reduced dendritic spine density in normal rodent hippocampus. Soluble Aβ from Alzheimer's disease brain also disrupted the memory of a learned behavior in normal rats. These various effects were specifically attributable to Aβ dimers. Mechanistically, metabotropic glutamate receptors were required for the LTD enhancement, and *N*-methyl D-aspartate receptors were required for the spine loss. Co-administering antibodies to the Aβ N-terminus prevented the LTP and LTD deficits, whereas antibodies to the midregion or C-terminus were less effective. Insoluble amyloid plaque cores from Alzheimer's disease cortex did not impair LTP unless they were first solubilized to release Aβ dimers, suggesting that plaque cores are largely inactive but sequester Aβ dimers that are synaptotoxic. We conclude that soluble Aβ oligomers extracted from Alzheimer's disease brains potently impair synapse structure and function and that dimers are the smallest synaptotoxic species.

Alzheimer's disease is distinguished histopathologically from other dementias by abundant extraneuronal deposits of Aβ. Numerous reports describe neuronal alterations induced by supraphysiological concentrations of synthetic Aβ peptides, by Aβ species secreted by cultured cells, or by complex mixtures of Aβ assembly forms in the brains of amyloid precursor protein (APP) transgenic mice[1–5]. Although these findings show that Aβ can alter synapse physiology in experimental models, the nature of the pathogenic species in the human brain and direct demonstration of its neurobiological effects are unresolved.

To extract and characterize Aβ present in human brain, we prepared aqueously soluble (Tris-buffered saline (TBS)), detergent-soluble (TBS with 1% Triton X-100) and 'insoluble' (5 M GuHCl) extracts by sequential centrifugation of brain homogenates from humans with various neuropathologically confirmed dementias (**Supplementary Table 1a** online). Immunoprecipitation and western blotting[5,6] revealed Aβ monomers and lithium dodecylsulfate (LDS)-stable dimers and trimers in all three extracts of the frontal and temporal cortices from subjects with Alzheimer's disease and an adult with Down's syndrome and Alzheimer's disease (**Fig. 1**). Cortical extracts from some subjects without Alzheimer's disease showed modest levels of Aβ in the insoluble (GuHCl) extracts (**Fig. 1c**) but little or none in the soluble (TBS) extracts (**Fig. 1a**) compared to the Alzheimer's disease cases. Notably, a subject with Alzheimer's disease histopathology but no clinical Alzheimer's disease (pathological Alzheimer's disease, P-AD) showed Aβ in the insoluble but not the soluble fraction (**Fig. 1**). Although Aβ was detectable in all three sequential extracts, we chose to characterize the physiologic effects of the TBS-soluble fraction because Alzheimer's disease dementia correlates strongly with soluble Aβ abundance[7–9]. Indeed, the profile of our extracts suggested that levels of TBS-soluble Aβ correlated best with the clinical Alzheimer's disease state (**Fig. 1a,c**). Moreover, we wished to focus on the earliest Aβ assemblies: soluble oligomers that form initially from monomers.

We first asked whether soluble Aβ from Alzheimer's disease cortex (**Fig. 2a** and **Supplementary Table 1b**) alters LTP in mouse hippocampus. TBS extracts from control (Con TBS) or Alzheimer's disease (AD TBS) cortex did not alter basal synaptic transmission or paired-pulse ratio (**Supplementary Fig. 1a,b** online), indicating that neurotransmitter release probability was unaffected[10]. Slices exposed to TBS vehicle (Veh) or Con TBS for 20 min showed robust LTP induction after high-frequency stimulation (HFS) (152.9 ± 9.1% and 144.2 ± 7.1% of baseline standard field excitatory postsynaptic potentials (fEPSP) slope, respectively; **Fig. 2b**). In contrast, AD TBS inhibited LTP (111.3 ± 3.9%, $P < 0.05$; **Fig. 2b**). Immunodepleting AD TBS with an antiserum to Aβ (R1282) prevented the LTP inhibition

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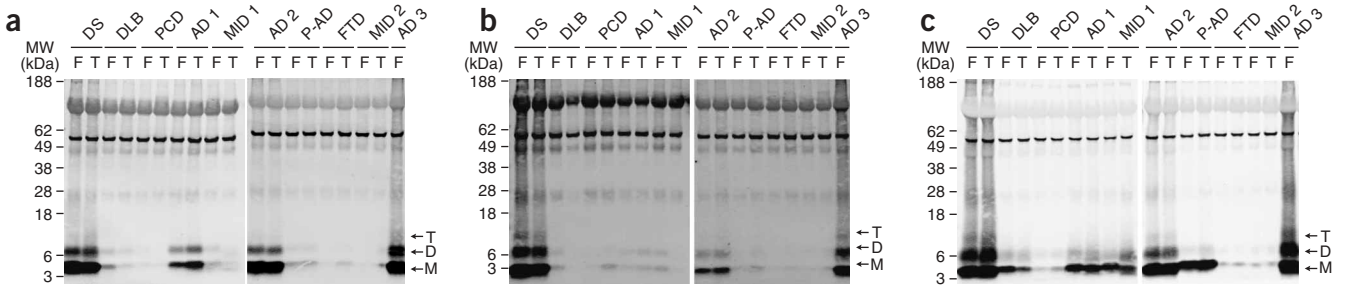

Figure 1 Monomeric and oligomeric Aβ is detected in brain extracts of humans with clinically and neuropathologically typical late-onset Alzheimer's disease. (**a**–**c**) Immunoprecipitation and western blotting (IP-WB) analysis was performed on supernatants of the soluble (**a**, TBS), membrane-associated (**b**, TBS-TX100) and insoluble (**c**, GuHCl) sequential extracts of frontal (F) and temporal (T) cortex homogenates from various individuals diagnosed with different forms of dementia (see **Supplementary Table 1**). Samples were immunoprecipitated with polyclonal antibody R1282 to Aβ and blotted with monoclonal antibodies 2G3 (to $Aβ_{40}$) and 21F12 (to $Aβ_{42}$). DS, Down's syndrome with Alzheimer's disease; DLB, dementia with Lewy bodies; PCD, paraneoplastic cerebellar degeneration; AD, Alzheimer's disease; MID, multi-infarct dementia; P-AD, pathological Alzheimer's disease (scattered amyloid plaques without a history of clinical Alzheimer's disease); FTD, frontotemporal dementia.

(**Fig. 2c**), indicating that Aβ was necessary for the inhibition. The effect of AD TBS on LTP was strongly dose dependent (**Supplementary Fig. 1d**). Notably, TBS extracts prepared identically from the cortices of individuals with frontotemporal dementia or dementia with Lewy bodies did not significantly alter LTP (137.0 ± 5.3% and 148.1 ± 6.1%, respectively) (**Fig. 2d** and **Supplementary Fig. 1e**). Additional brain extracts from two control subjects and three subjects with Alzheimer's disease fully replicated the above findings (**Fig. 2a**,**d**).

LTD of hippocampal synapses is induced by persistent subthreshold stimulation[11]. Standard protocols for LTD induction in adult rodent hippocampus require delivery of 600–900 pulses at low frequency[12,13]. Accordingly, 300 pulses at 1 Hz failed to induce LTD in the presence of vehicle or Con TBS (**Fig. 2e**). However, AD TBS facilitated LTD induction by this weak stimulus (74.7 ± 4.8% of baseline for AD TBS versus 101.9 ± 5.6% for Con TBS, $P < 0.05$; **Fig. 2e**). LTD induced with AD TBS was *N*-methyl D-aspartate receptor (NMDAR) independent, as the NMDAR antagonist AP-V did not block this effect (68.1 ± 4.3%; **Fig. 2f**). However, both α-methyl-4-carboxyphenylglycine (MCPG), a group I/II metabotropic glutamate receptor (mGluR) antagonist (94.8 ± 2.4%, $P < 0.05$), and SIB1757, an mGluR5 antagonist (101.1 ± 6.9%, $P < 0.05$), prevented LTD facilitation by AD TBS (**Fig. 2f**). Whereas mGluR activation was necessary for the LTD facilitation by soluble Aβ, SIB1757 did not prevent AD TBS–mediated LTP inhibition (**Supplementary Fig. 1f**). This finding is consistent with earlier data showing that Aβ can influence synaptic plasticity through various receptors, including NMDAR, mGluR and nicotinic acetylcholine receptors[14–17].

Passive administration of monoclonal Aβ antibodies has entered human testing for treatment of Alzheimer's disease. We found that the ability of a co-administered Aβ antibody to block the above-mentioned LTD facilitation correlated with its ability to immunoprecipitate soluble Aβ from AD TBS (**Supplementary Fig. 2** online). Antibodies to the free N-terminus of Aβ (3D6; 82E1) almost completely precipitated soluble Aβ from AD TBS and also prevented the LTD facilitation (98.4 ± 3.0%), whereas antibodies to the Aβ C-terminus (2G3, 21F12) weakly precipitated Aβ and did not block the LTD effect (72.1 ± 4.9%; **Supplementary Fig. 2a**,**b**). Aβ midregion antibodies immunoprecipitated a fraction of the Aβ species in AD TBS and only partially blocked the LTD effect (**Supplementary Fig. 2a**,**c**). Similarly, N-terminal but not C-terminal antibodies neutralized the LTP deficit (**Supplementary Fig. 2d**).

To assess the effects of soluble Alzheimer's disease cortical extracts directly on memory function, we trained rats on a step-through passive avoidance task[18]. At 0, 3 or 6 h after training, we injected AD TBS or R1282-immunodepleted AD TBS (AD TBS-ID) (**Supplementary Fig. 3a** online) into the lateral ventricle. AD TBS administered 3 h after training significantly impaired the rats' recall of the learned behavior 48 h later, such that the latency to enter the dark chamber, where the rat had received a shock during training, was significantly ($P < 0.05$) shorter for rats injected with AD TBS than with AD TBS-ID (**Fig. 2g**). Notably, AD TBS injected at 0 or 6 h after training did not significantly alter the escape latency (**Supplementary Fig. 3**). The 3-h post-training time point at which AD TBS significantly impaired recall is consistent with the temporal pattern of transcriptional regulation of synapse remodeling following passive avoidance training[19].

Decreased synapse density is the strongest neuropathological correlate of the degree of dementia in Alzheimer's disease[20]. To determine whether soluble Aβ in Alzheimer's disease brain contributes directly to synapse loss, we quantified dendritic spine density in GFP-transfected pyramidal cells in organotypic rat hippocampal slices[21]. To properly reconstitute brain extracts in slice culture medium, we subjected TBS extracts to nondenaturing size-exclusion chromatography (SEC). Pyramidal neurons in slices cultured for 10 d with plain medium (sham) or medium reconstituted with lyophilized SEC fractions of Con TBS (Con TBS-SEC) showed similar spine densities (0.79 ± 0.02 and 0.86 ± 0.03 spines/μm, $n = 6/890$ and 5/628 cells/spines, respectively). In contrast, slice medium reconstituted with SEC fractions from AD TBS (AD TBS-SEC) caused a 47% decrease in spine density versus Con TBS-SEC (0.46 ± 0.03 spines/μm, $P < 0.05$; $n = 6/517$; **Fig. 2h** and **Supplementary Fig. 4** online). MCPG did not prevent the loss of spines with AD TBS-SEC treatment (0.45 ± 0.03 spines/μm; $n = 5/337$; **Fig. 2h**). 3-((*R*)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), an NMDAR antagonist, did not alter spine density when applied alone but prevented the decrease observed with AD TBS-SEC (0.73 ± 0.03 and 0.84 ± 0.03, respectively; $n = 5/619$ and 5/748; $P < 0.05$ for AD TBS-SEC alone versus AD TBS-SEC with CPP; **Fig. 2h**). These findings support previous evidence that NMDAR activation is necessary for Aβ-mediated spine loss[16,17].

We next asked which soluble Aβ species present in Alzheimer's disease brain mediated these effects on synapse physiology. Two lines of evidence indicated that the Aβ-immunoreactive species migrating

at 8 kDa on LDS-PAGE gels were true Aβ dimers. First, mass spectrometry of the 4- and 8-kDa bands immunoprecipitated from the GuHCl extract of Alzheimer's disease cortex confirmed that each contained tryptic peptides of human Aβ (**Supplementary Fig. 5b–e** online). Second, immunoprecipitation of this extract with an $Aβ_{40}$-specific antibody (2G3) and western blot analysis with an $Aβ_{42}$-specific antibody (21F12) revealed a heterodimer composed of $Aβ_{40}$ and $Aβ_{42}$ migrating at 8 kDa (**Supplementary Fig. 5a**). Performing this coimmunoprecipitation with 21F12 for both the immunoprecipitation and the western blot yielded a much stronger dimer signal, indicating that most of the 8-kDa species are $Aβ_{40}$–$Aβ_{42}$ homodimers (**Supplementary Fig. 5a**).

Having confirmed that the 8-kDa bands detected by western blotting in Alzheimer's disease brain samples (**Figs. 1** and **2a**) are *bona fide* Aβ dimers, we used nondenaturing SEC to separate the various Aβ species in AD TBS and characterized their respective effects on LTP. Most of the Aβ in AD TBS eluted in the void volume (fractions 3–4, >60 kDa based on co-eluting linear polydextran standards[22]), but this higher molecular weight complex dissociated into Aβ monomers and dimers when denatured by LDS-PAGE (**Fig. 3a**). This SEC profile also showed dimers eluting at ~8–16 kDa (fractions 7–8) and monomers eluting at ~3–6 kDa (fractions 10–11). Taken together, these results indicate that in Alzheimer's disease cortex, soluble Aβ exists in various assemblies, with the smallest native oligomer being a dimer.

To establish which soluble Aβ species were responsible for the impaired synaptic plasticity, SEC fractions of AD TBS containing either higher molecular weight complexes (AD SEC 4), native Aβ dimers (AD SEC 8) or monomers (AD SEC 10) were each tested separately. Only AD SEC 8 significantly inhibited LTP (107.0 ± 2.2%; $P < 0.05$ versus Con TBS), whereas AD SEC 4, AD SEC 10 and identically prepared fractions from Con TBS were all inactive (**Fig. 3b**). Notably, the AD SEC 4 fraction contained the highest concentration of Aβ (**Fig. 3a**), suggesting that the specific activity of this higher molecular weight Aβ assembly is very low. To achieve a purer preparation of Aβ dimers, AD TBS was immunoprecipitated with 3D6, eluted with denaturing LDS buffer and subjected to SEC (IP-SEC; **Fig. 3c**). Most of the soluble Aβ now eluted at the size of dimers (fractions 7–8) rather than in the void volume (**Supplementary Fig. 6** online), suggesting that elution with LDS disrupts noncovalent interactions among the higher molecular weight Aβ assemblies. LTP was significantly inhibited by IP-SEC fractions 7–8, containing Aβ dimers (106.6 ± 2.4%, $P < 0.05$), but not by fractions 10–11 containing monomers nor by any IP-SEC fractions from Con TBS (**Fig. 3d**).

Although these SEC experiments showed that soluble Aβ dimers inhibit LTP, it remained possible that a small molecule from human brain was bound to the Aβ dimers and was required to impair LTP. To address this possibility, we generated a synthetic $Aβ_{40}$ peptide in which Ser26 was mutated to cysteine ($Aβ_{40}$-S26C). An Aβ dimer was observed upon oxidation (**Fig. 3e**), and this inhibited LTP nearly 20 times more potently than did wild-type synthetic $Aβ_{40}$ (**Fig. 3f**). This pure, synthetic dimer cannot contain any other factors present in AD TBS, establishing that Aβ dimers alone are sufficient to perturb synapse physiology.

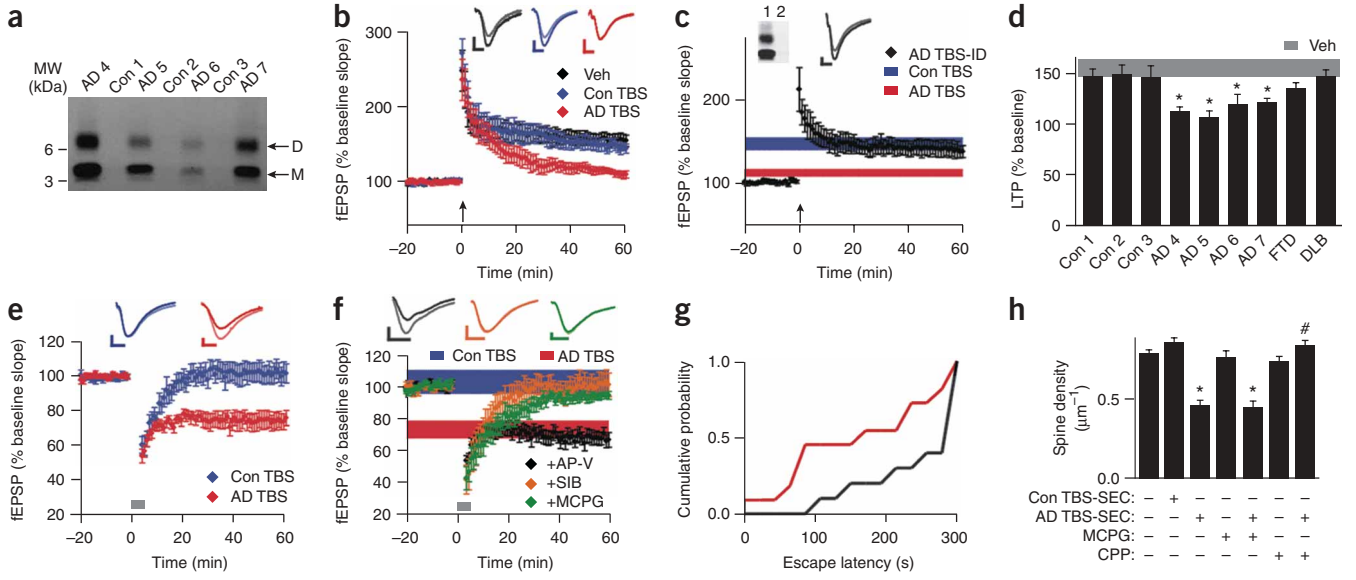

Figure 2 Soluble Aβ extracted from Alzheimer's disease brain alters hippocampal synapse physiology and learned behavior. (**a**) IP-WB of the TBS brain extracts used to study LTP. (**b**) Summary data of LTP induction after treatment with TBS vehicle (Veh; $n = 8$ slices), Con 1 TBS extract (Con TBS; $n = 6$) or AD 4 TBS extract (AD TBS; $n = 8$). Insets show average baseline (light) and post-HFS (dark) fEPSP traces; calibration bars 5 msec/0.2 mV. (**c**) LTP is induced normally in hippocampal slices treated with immunodepleted AD TBS (AD TBS-ID). The summary LTP data for Con TBS (blue, $n = 6$ slices) and AD TBS (red, $n = 8$ slices) from **b** are represented as horizontal bars depicting means ± s.e.m. Inset, two sequential immunoprecipitations (R1282) of AD TBS. (**d**) Summary LTP data (means ± s.e.m.) for three different control subjects (Con 1, $n = 6$; Con 2, $n = 6$; Con 3, $n = 6$), four subjects with AD (AD 4 $n = 8$; AD 5, $n = 5$; AD 6, $n = 6$; AD 7, $n = 6$), one subject with FTD ($n = 5$) and one subject with DLB ($n = 6$). For comparison, LTP data from **b** for Veh are represented by the gray horizontal bar. $^*P < 0.05$ compared with Veh. (**e**) Summary LTD data for slices treated with Con TBS (blue, $n = 7$) or AD TBS (red, $n = 8$) before stimulating with 300 pulses at 1 Hz, indicated by the small gray bar. Calibration bars, 5 msec/0.2 mV. (**f**) Summary LTD data for co-administration of AD TBS with either 50 μM AP-V (black, $n = 8$), 500 μM (R/S)-MCPG (green, $n = 7$) or 3 μM SIB1757 (orange, $n = 6$). For comparison, LTD data (means ± s.e.m.) for Con TBS (blue) and AD TBS (red) are shown as horizontal bars. (**g**) Rats receiving AD TBS (red) had a significantly shorter mean escape latency than animals receiving AD TBS-ID (black) at 48 h after training (174 ± 31.7 s and 255 ± 22.8 s, respectively; $P < 0.05$; $n = 11$ and 10 rats). (**h**) Summary spine density data for pyramidal cells exposed to AD TBS-SEC or Con TBS-SEC, and also for slices treated with 500 μM MCPG or 20 μM CPP in the presence or absence of AD TBS-SEC. * $P < 0.05$ and $^{\#}P < 0.05$ versus Con TBS-SEC and versus AD TBS-SEC, respectively.

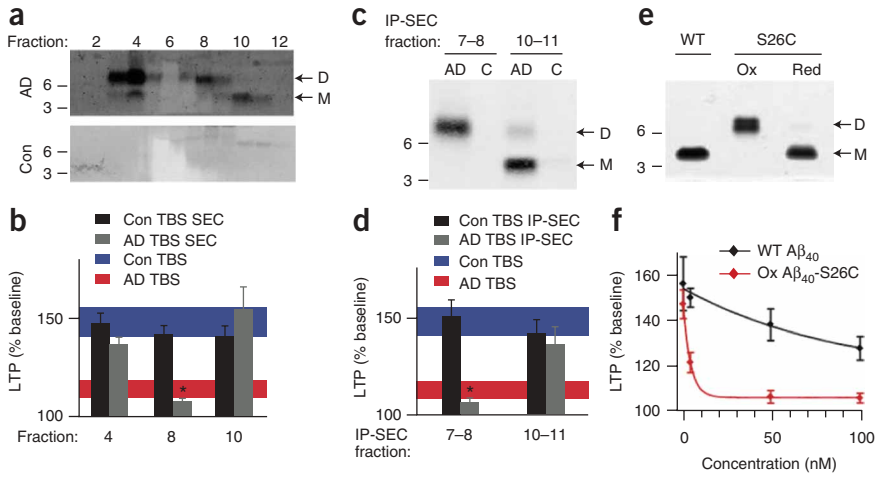

Figure 3 Soluble dimers are the smallest Aβ assembly form in human brain to acutely perturb synapse physiology. (**a**) AD TBS (top) and Con TBS (bottom) were subjected to nondenaturing SEC and blotted with 2G3 and 21F12. Aβ monomer (M) and dimer (D) in AD TBS is recovered in the void volume (fractions 3 and 4). The white areas at the bottom of lanes 5–7 are intrinsic to these gels and represent an unknown soluble factor that we commonly observe in western blots of SEC fractions of human brain TBS extracts and that interferes with blocking, antibody labeling or both. (**b**) Summary LTP data (means ± s.e.m.) for slices treated with SEC fractions characterized in **a** ($n = 6$ slices for all samples). (**c**) IP-SEC fractionation of AD TBS generates dimer-enriched (fractions 7–8) and monomer-enriched (fractions 10–11) fractions. (**d**) Summary LTP data (means ± s.e.m.) for slices treated with IP-SEC fractions of AD and Con TBS brain extracts, as characterized in **c** (Con TBS fractions 10–11, $n = 5$; AD TBS fractions 10–11, $n = 5$; Con TBS fractions 7–8, $n = 7$; AD TBS fractions 7–8, $n = 7$). (**e**) Silver stain showing that mutant $A\beta_{40}$-S26C forms dimers when oxidized (ox) and can be reduced (red) to monomers with β-mercaptoethanol treatment. (**f**) Summary LTP data (means ± s.e.m.) for slices treated with either WT $A\beta_{40}$ (black) or oxidized $A\beta_{40}$-S26C (red) reveals that the oxidized $A\beta_{40}$-S26C dimer inhibits LTP with much greater potency (100 nM $A\beta_{40}$-S26C, $n = 4$; $n = 5$ for all other treatments). The vehicle controls (plotted at 0 nM) were 50 mM ammonium acetate ($n = 4$) for the S26C peptide and 0.1% ammonium hydroxide ($n = 4$) for the WT peptide.

Previous studies have suggested that unlike soluble Aβ abundance, amyloid plaque burden correlates poorly with Alzheimer's disease severity[7,9,20]. We asked whether insoluble amyloid cores isolated from Alzheimer's disease cortex can inhibit hippocampal LTP. To isolate these detergent-resistant foci of fibrillar Aβ from neuritic plaques[23–25], we homogenized TBS-insoluble pellets of plaque-rich Alzheimer's disease cortex in 2% SDS[24]. Immunoprecipitation and western blotting of supernatants after washing in SDS buffer showed that no additional Aβ was liberated by SDS or TBS from this preparation (**Fig. 4a**). Congo red staining of the residual pellet revealed intact amyloid cores showing characteristic birefringence (**Fig. 4b**). Although resistant to disruption by many solvents, Alzheimer's disease amyloid cores are efficiently solubilized by formic acid[23,24]. This treatment released Aβ dimers and monomers from the washed cores (**Fig. 4a**). When we applied this formic acid extract of the Alzheimer's disease core preparation to hippocampal slices, LTP was inhibited (116.2 ± 4.6%, $P < 0.05$ versus formic acid vehicle; **Fig. 4c**). Formic acid extracts of identically prepared fractions from control brain allowed normal LTP (**Fig. 4c**). Thus, amyloid cores contain Aβ dimers that can impair synaptic plasticity. In contrast, addition of intact cores (**Fig. 4b**) to the artificial cerebrospinal fluid (ACSF) perfusate did not affect LTP (139.4 ± 7.6%, **Fig. 4d**). Therefore, in physiologic buffer (ACSF), amyloid cores do not acutely release soluble Aβ dimers to alter synaptic plasticity. Immunoprecipitation and western blot analysis revealed that Aβ dimers also were not released from amyloid cores incubated in physiological buffers at 37 °C for 24 h (**Supplementary Fig. 7** online), suggesting that highly insoluble Aβ aggregates such as amyloid plaque cores represent dimer-rich structures that do not readily dissociate.

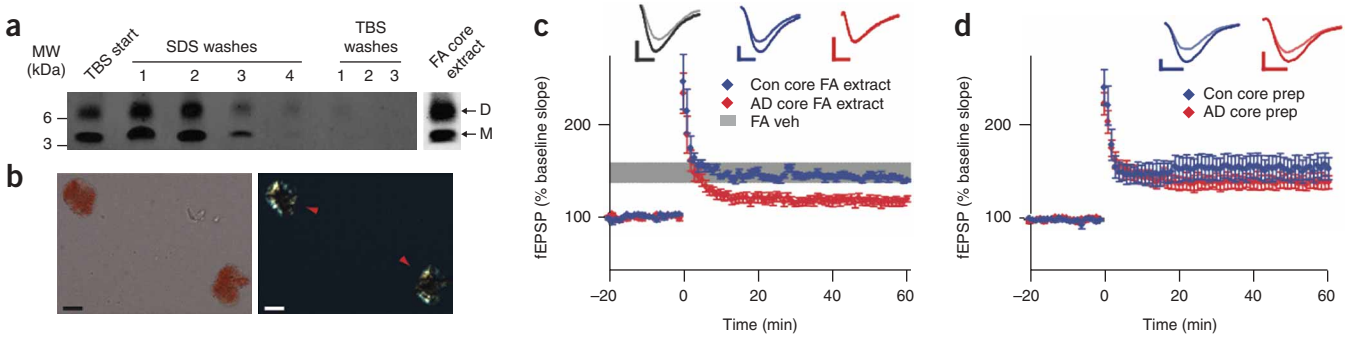

Figure 4 Insoluble amyloid cores contain Aβ dimers with synaptotoxic potential but are not readily released. (**a**) IP-WB of sequential extracts of the TBS-insoluble pellet prepared from 100 mg of a plaque-rich Alzheimer's disease brain (case AD 5 from **Fig. 2a**). The final TBS washes reveal that no additional soluble Aβ can be extracted from the pellet after four sequential SDS washes. The remaining core-rich pellet was then incubated in formic acid (FA core extract) and analyzed by IP-WB, revealing that the insoluble cores contain Aβ monomers and dimers (far right lane). (**b**) Core preparations after the final TBS wash in **a** were stained with 0.2% Congo red and visualized by brightfield (left) and polarization (right) microscopy. Isolated amyloid cores show characteristic birefringence with Congo red (red arrowheads). Material prepared similarly from Con 3 (**Fig. 2a**) did not contain any such structures. Scale bar, 5 μm. (**c**) Cores prepared as in **a** and **b** were extracted with 88% formic acid and neutralized with NaOH. Summary LTP data for slices treated with just FA and NaOH vehicle (FA Veh, $n = 5$), or with FA and NaOH core extracts from Alzheimer's disease (AD core FA extract, $n = 7$) or control (Con core FA extract, $n = 5$) brains. Calibration bars, 5 msec/0.2 mV. (**d**) Summary LTP data for slices exposed to intact core preps isolated as in **a** and **b** from 100 mg Alzheimer's disease cortex (AD core prep, $n = 5$) or Con cortex (Con core prep, $n = 5$). Calibration bars, 5 msec/0.3 mV.

Here we show that soluble Aβ isolated directly from Alzheimer's disease brains potently and consistently induces several Alzheimer's disease–like phenotypes in normal adult rodents: it decreases dendritic spine density, inhibits LTP and facilitates LTD in hippocampus, and interferes with the memory of a learned behavior. We used nondenaturing gel filtration coupled with immunoprecipitation and western blotting and subsequent immunodepletion or neutralization with epitope-specific antibodies to Aβ to ascribe the pathogenic effects to soluble Aβ oligomers, principally dimers.

Our findings support the emerging concept that the effects of Aβ in Alzheimer's disease initially center on subtly altered synapse function. Neither Aβ monomers nor insoluble amyloid plaque cores significantly altered synaptic plasticity. This does not mean that insoluble amyloid plaques have no pathogenic role; their invariant accumulation may signify that they serve as relatively inert reservoirs of small bioactive oligomers, and they may disassemble more readily in the presence of lipids[26]. That plaque cores may release locally active Aβ species *in vivo* is suggested by a penumbra of synapse loss around cores in APP transgenic mice[27].

Our examination of soluble dimers obtained from Alzheimer's disease brain is partially consistent with findings using synthetic[2] or cell-derived[5,28] Aβ oligomers. However, there are unresolved differences regarding the precise biochemical nature of the synaptotoxic species found in these various systems. For example, we did not detect in human cortical extracts (**Fig. 1** and **Supplementary Fig. 6**) a soluble, SDS-stable dodecamer of Aβ, similar to the Aβ*56 species observed in brain extracts from certain APP transgenic mice[29]. Soluble Aβ complexes from Alzheimer's disease cortex eluted in the void volume (>70 kDa) upon nondenaturing SEC, but these dissociated into dimers and monomers upon LDS-PAGE. Some Alzheimer's disease and aged control cerebrospinal fluid samples that contain soluble Aβ dimers were recently shown to impair LTP[30], a finding consistent with our data. However, the invariant detection of dimers in the soluble fraction of Alzheimer's disease cortex and their multiple synaptic effects strongly suggest that cortical dimers contribute directly to synapse dysfunction in people with Alzheimer's disease, whereas any additional effects of cerebrospinal fluid dimers in the minority of Alzheimer's disease subjects who have them[30] remain to be determined.

Mechanistically, we show that soluble Aβ dimers from Alzheimer's disease cortex induce their effects by perturbing glutamatergic synaptic transmission. Although we find that mGluRs are required for the induction of LTD, whereas NMDARs are needed for spine loss, these receptors are unlikely to be the sole effector targets of soluble Aβ oligomers. Aβ extracted from human brain can now serve as the most pathophysiologically relevant material for further pathway analysis and for preclinical validation of agents designed to neutralize Aβ aggregates. Our findings fulfill an essential requirement for establishing disease causation in Alzheimer's disease.

METHODS

Human brain sample preparation. We collected brain specimens from deceased human subjects at autopsy after obtaining informed consent from the next of kin under protocols approved by the Partners Human Research Committee at Brigham and Women's Hospital and the Ethics Review Committee/Institutional Review Board at Beaumont Hospital in Dublin, Ireland. Each subject's clinical and neuropathological diagnoses are provided in **Supplementary Table 1** online. We weighed frozen human temporal or frontal cortices containing white and gray matter, added freshly prepared, ice-cold TBS consisting of 20 mM Tris-HCl, 150 mM NaCl, pH 7.4 to the frozen cortex at 4:1 (TBS volume / brain wet weight) and homogenized with 25 strokes at a setting of 10 on a mechanical Dounce homogenizer. We spun the homogenate at 175,000*g* in a TLA100.2 rotor on a Beckman TL 100 centrifuge. We aliquoted and then stored the supernatant (called TBS extract) at –80 °C, and we rehomogenized the pellet (4:1 vol/wt) in TBS plus 1% Triton X-100 and spun as above. The resultant supernatant (called TBS-TX extract) was aliquoted and stored at –80 °C, and the pellet was rehomogenized in TBS plus 5 M guanidine HCl, pH 8.0, and incubated on a Nutator for 12–16 h at 22°C; the resultant supernatant (GuHCl extract) was aliquoted and stored at –80 °C.

Size exclusion chromatography. We injected 1-ml aliquots of the TBS-soluble Alzheimer's disease brain extract onto a Superdex 75 (10/30HR) column (Amersham Biosciences) and eluted at a flow rate of 1 ml/min into 1-ml SEC fractions using 50 mM ammonium acetate, pH 8.5. We removed 750 µl and stored it at –80 °C. We lyophilized the remaining 250 µl, reconstituted it in 15 µl of 2× LDS sample buffer, heated it at 70 °C for 5 min and electrophoresed it on a 26-well 4–12% bis-Tris gel using MES running buffer (Invitrogen). We transferred proteins to 0.2-µm nitrocellulose and western blotted for Aβ with 1 µg/ml 2G3 and 21F12 (gifts of Elan) using the LiCor Odyssey Infrared Imaging System. We polled SEC fractions containing higher order Aβ assemblies, LDS-stable Aβ dimers or Aβ monomers separately before lyophilizing them into 450-µl aliquots.

Immunoprecipitation and western blotting analysis of Aβ in human brain extracts. We used an immunoprecipitation and western blotting protocol described previously[6] to detect Aβ in the TBS, TBS-TX and GuHCl extracts. We immunoprecipitated TBS extracts directly with either polyclonal antiserum R1282 to Aβ (1:50) plus Protein A sepharose (PAS; Sigma) or monoclonal antibodies to Aβ 3D6 (3 µg/ml) or 2G3 and 21F12 (each at 3 µg/ml; gifts of Elan) plus Protein G agarose (PGA; Roche) and PAS. We diluted GuHCl extracts 1:40 in DMEM and then immunoprecipitated with R1282 (at 1:50) and PAS or with 2 µg/ml 266, 2G3 and 21F12 and PGA and PAS. We used the SilverQuest kit fast protocol (Invitrogen) for silver staining.

Hippocampal slice electrophysiology recording. The Harvard Medical School Standing Committee on Animals approved all experiments involving mice and rats used for electrophysiology and dendritic spine analysis. We recorded fEPSP in the CA1 region of the mouse hippocampus. We placed a unipolar stimulating electrode (World Precision Instruments) in the Schaffer collaterals of CA3 neurons to deliver test and conditioning stimuli. We positioned a borosilicate glass recording electrode filled with ACSF in stratum radiatum of CA1 200–300 µm from the stimulating electrode. We induced fEPSPs in CA1 by two test stimuli at 0.05 Hz with an intensity that elicited a fEPSP amplitude ~40–50% of maximum. Once we had attained a stable test response for at least 30–60 min, we added experimental treatments to the 9.5-ml ACSF perfusate and recorded a baseline for an additional 20 min. These treatments included: 500 µl TBS extract, 500 µl TBS vehicle, 50 µM AP-V, 500 µM (R/S)-MCPG (Tocris) and 3 µM SIB1757. The lyophilized 450-µl aliquots of SEC fractions of GuHCl extracts described above were reconstituted in 500 µl ACSF and added to the slice perfusates. To induce LTP, we applied two consecutive trains (1 s) of stimuli at 100 Hz separated by 20 s, a protocol that induced LTP lasting approximately 1.5 h in wild-type mice of this genetic background. To induce LTD, we delivered 300 pulses at 1 Hz. We amplified the field potentials 100× with an Axon Instruments 200B amplifer and digitized with Digidata 1322A. We sampled the data at 10 kHz and filtered them at 2 kHz. We obtained traces by pClamp 9.2 and analyzed them with the Clampfit 9.2 program. The LTP and LTD values reported throughout were measured at 60 min after the conditioning stimulus unless stated otherwise. We monitored paired-pulse responses at 50-ms inter-stimulus intervals. We calculated the facilitation ratio as fEPSP2 slope/fEPSP1 slope.

Passive avoidance conditioning. We performed passive avoidance training as described previously[18] (see **Supplementary Methods** online). We gave Wistar rats AD TBS or AD TBS immunodepleted with R1282 at 0, 3 or 6 h after training. We evaluated recall of the passive avoidance conditioning 24 and 48 h after training by recording the latency to enter the dark chamber, with a criterion time of 300 s.

Dendritic spine density analysis. We prepared, treated, imaged and analyzed the apical dendrites of pyramidal cells in organotypic hippocampal slices as

previously described[17] (see **Supplementary Methods**). We treated slices for 10 d with AD TBS-SEC or Con TBS-SEC. We performed pharmacologic treatments with 20 μM D-CPP or 500 μM (R/S)-MCPG in the presence or absence of AD TBS-SEC for 10 d.

Statistical analyses. Data from the electrophysiology and passive avoidance experiments were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni *post-hoc* test to determine statistical significance. Dendritic spine morphology data were analyzed by one-way ANOVA with Tukey-Kramer's correction for multiple pairwise comparisons.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

G.M.S. designed and performed experiments and prepared the manuscript; S.L. designed and performed electrophysiology experiments; T.H.M. and N.E.S. performed biochemical experiments; A.G.-M. performed passive avoidance experiments; I.S. generated mutant Aβ peptide; C.A.L., F.M.B. and M.A.F. characterized human brain tissue; M.J.R. designed electrophysiology experiments; C.M.R. designed passive avoidance experiments; D.M.W. designed biochemical experiments; B.L.S. designed electrophysiology and morphology experiments; and D.J.S. designed experiments and prepared the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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