Supporting Information

Balducci et al. 10.1073/pnas.0911829107

SI Text

**Aβ$_{1-42}$ Synthesis and Sample Preparation.** Densi-peptide Aβ$_{1-42}$ (1) was synthesized in a mainly automated manner using an ABI 433A synthesizer (Applied Biosystems) on TentaGel-resin (Novabiochem) with a 10-fold excess of Fmoc-protected L-amino acids and 2- (6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate as coupling reagent (2). Only the densi-peptide Boc-Ser(Fmoc-Gly) (Novabiochem) was manually introduced after position 27, using a filter fitted syringe (3). Afterward, the amino acids were again coupled using the automated synthesizer. The peptide was cleaved from the resin with 0.3 g phenol in a H2O/THF/EDT/TFA (water/thioglycerol/thiophenol/trifluoroacetic acid) (0.2 mL/0.2 mL/0.1 mL/4 mL) solution for 3 h, poured into ice-cold diethyl ether, filtered, washed three times with ice-cold diethyl ether, and dried under vacuum for 20 min. The peptide was purified by HPLC equipped with a C4 column (Water Symmetry 19 × 150 mm). The molecular mass of the final product was determined by MALDI-TOF spectrometry (Bruker).

Densi-Aβ$_{1-42}$ was dissolved in an acidic solution (TFA 0.02%, pH <3) at a concentration of ~1 mg/mL and centrifuged through a 10-kDa cut-off filter (YM-10, Millipore) at 14,000 × g. The filter was washed twice by addition of same acidic solution, and the centrifugation was repeated. This stock solution was flash-frozen in dry ice/ethanol and stored at −80 °C. Samples were used within 2 weeks. The native sequence was obtained by adding 0.5 M NaOH/ammonium (1:3) solution on ice to pH >10.5. This solution was kept for 15 min on ice. The concentration of the native peptide in the final, washed, alkaline stock solution was 300 μM. This was diluted to 1 μM Aβ in PBS and used immediately (“initial state solution”). To obtain Aβ$_{1-42}$ oligomers, the stock solution was diluted to 100 μM Aβ in 50 mM phosphate buffer and 150 mM NaCl, pH 7.4, and incubated for 24 h at either 4 °C (4) or 22 °C (5). To prepare Aβ$_{1-42}$ fibrils, the stock solution was diluted with water to 100 μM Aβ, acidified to pH 2.0 with 1 M HCl, and left for 24 h at 37 °C (6).

**Size Exclusion Chromatography.** Size exclusion chromatography (SEC) was performed on an FPLC apparatus (Biologic FPLC system, Biorad) equipped with a precision column prepacked with Superdex 75 resin, with a separation range of 3–70 kDa (GE Healthcare). The mobile phase flow rate was set at 0.5 mL/min, and the elution peaks were detected at 214 and 280 nm UV absorbance. The mobile phase was 25 mM phosphate buffer (PBS, pH 7.4). The column was calibrated using insulin chain B (3.5 kDa), ubiquitin (8.5 kDa), ribonuclease A (13.7 kDa), carbonate anhydrase (29.0 kDa), ovalbumin (43.0 kDa), and BSA (67.0 kDa). The void volume was determined by Blue dextran 2000 (2,000 kDa), and each peptide solution was injected at a flow rate of 0.5 mL/min, and each peptide solution was injected at a concentration of 20 μM in a volume of 100 μL.

**Surface Plasmon Resonance and Aβ$_{1-42}$ Oligomer Binding to PrP$^C$.** Binding studies were done with the ProteOn XPR36 Protein Interaction Array system (Bio-Rad), based on surface plasmon resonance (SPR) technology. The system contains six parallel-flow channels, which can uniformly immobilize up to six strips of ligands on the sensor surface (7). Two anti-PrP antibodies, 3F4 (8) against epitopes 108–111, and 94B4 (9) against epitopes 187–194 (mouse PrP numbering) were immobilized in two parallel lanes, using amine-coupling chemistry, with final immobilization levels of ~6,000 resonance units (RU, 1 RU = 1 pg protein/mm²) for both. “Reference” surfaces were prepared in parallel following the same immobilization procedure but without anti-K$_d$ values of the antibody.

**Animals.** C57BL/6 mice were obtained from Charles River-Italy. Zürich 1 Prnp$^{0/0}$ mice (11) maintained on a pure C57BL/6 background were obtained from the European Mouse Mutant Archive (strain EM01723). Animals were 7–8 weeks old. All animals were handled for 2 days before the experiments. Based on the previous experience, we used only males (12). All procedures involving animals and their care were conducted according to European Union (EEC Council Directive 86/609, OJ L 358,1; 12 December 1987) and Italian (D.L. n.116, G.U. suppl. 40, 18 February 1992) laws and policies, and in accordance with the United States Department of Agriculture Animal Welfare Act and the National Institute of Health (Bethesda, MD) policy on Humane Care and Use of Laboratory Animals.

**Intracerebroventricular Incubation.** Mice were anesthetized with Forane (Abbott) using stereotaxic apparatus (model 900, David Kopf) a 7 mm-long guide cannula was implanted into the cerebral lateral ventricle (L ± 1.0 and DV –3.0 from dura with incisor bar at 0°) and secured to the skull with two stainless steel screws and dental cement. To avoid infections the animals received i.p. injections of 150 mg/kg/day Amplital (Pfizer) for 3 consecutive days after surgery. Mice were allowed 10–15 days to recover from surgery before the experiment.

**Aβ$_{1-42}$ Treatment.** The Aβ$_{1-42}$ preparations, checked by AFM, were infused into the lateral cerebral ventricle using an injection unit inserted into the guide cannula. The Aβ$_{1-42}$ were diluted to 1 μM in 5 mM PBS, pH 7.4, and 7.5 μL were infused using a Hamilton syringe in a total time of 5 min. The injection unit was left in place for 2 min more to allow the liquid to diffuse.

**Object Recognition Task.** Mice were tested in an open-square gray arena (40 × 40 cm), 30 cm high, with the floor divided into 25 squares by black lines. The following objects were used: a black plastic cylinder (4 × 5 cm), a glass vial with a white cup (3 × 6 cm), and a metal cube (3 × 5 cm). The task started with a habituation trial during which the animals were placed in the empty arena for 5 min, and their movements were recorded as the
number of line crossings. The next day, mice were again placed in the same arena containing two identical objects (familiarization phase). Exploration was recorded in a 10-min trial by an investigator (C.B.) blinded to the strain and treatment. Sniffing, touching, and stretching the head toward the object at a distance not more than 2 cm were scored as object investigation.

Twenty-four hours later (test phase) mice were again placed in the arena containing two objects: one identical to one of the objects presented during the familiarization phase (familiar object), and a new, different one (novel object), and the time spent exploring the two objects was recorded for 10 min. Memory was expressed as a discrimination index, i.e., (seconds on novel − seconds on familiar)/(seconds on novel + seconds on familiar). Animals with no memory impairment spent longer investigating the novel object, giving a higher discrimination index. Mice were injected with Aβ1-42 samples or the vehicle (PBS) alone 2 h before the familiarization and test phases. At the end of the experiment the mice were killed and their brains were taken for histological analysis to verify the correct placement of the cannula.

Hippocampal Neuron Cultures and Determination of Aβ1-42 Oligomer Toxicity. Primary hippocampal cultures were prepared from mice 2 days of age. The dissected hippocampus was incubated with 200 units of papain (Sigma Aldrich) for 30 min at 34 °C, and with trypsin inhibitor (Sigma Aldrich) for 45 min at 34 °C before mechanical dissociation. Neurons were plated on 96-well plates (~5 × 10^4 cells/well) precoated with 25 μg/mL poly-D-lysine (Sigma Aldrich). The plating medium was B27/neurobasal (Life Technologies) supplemented with 0.5 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Twelve days from the plating date, the neurons were treated with either 1 or 3 μM synthetic Aβ1-42 oligomers prepared at both 4 °C and 22 °C. After 72 h of Aβ treatment, cell survival was measured by MTT assay.