Lipids revert inert Aβ amyloid fibrils to neurotoxic protofibrils that affect learning in mice

Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by neurofibrillary tangles and amyloid plaques consisting of aggregated Aβ-peptide (Hardy, 2002). Fifteen years ago, the ‘amyloid hypothesis’ for AD has been proposed (Selkoe, 1991; Hardy and Higgins, 1992), but the discrepancies between amyloid plaque load in the brain and cognitive impairment in the patient (Price and Morris, 1999) or mice (Games et al, 1995) have caused a lot of controversy in the field (Terry, 2001). This has led to the concept of ‘protofibrils’ (Harper et al, 1997; Walsh et al, 1997, 1999; Hartley et al, 1999), ‘annular assemblies’ (Lashuel et al, 2002; Bitan et al, 2003); ‘Aβ-derived diffusible ligands’ (Lambert et al, 1998) or ‘soluble toxic oligomers’ (Podlisny et al, 1995, 1998; McLean et al, 1999; Walsh et al, 2002a; Glabe and Kayed, 2006). These species are intermediary forms between free soluble Aβ-peptides and insoluble amyloid fibers and are toxic in vitro and in vivo, whereas mature Aβ-amyloid fibers are largely inert (Aksenov et al, 1996). The molecular nature of these smaller assemblies of Aβ remains rather elusive (Hepler et al, 2006), as different sources, isolation procedures and biophysical techniques lead to different conclusions. A number of species have been observed: dimeric and trimeric Aβ-oligomers (Podlisny et al, 1995, 1998; McLean et al, 1999; Walsh et al, 2002a), 56 kDa oligomer assemblies from transgenic mouse brains (Lesne et al, 2006) or larger structures that consist of 50 and more Aβ peptides (Lambert et al, 1998; Walsh et al, 1999; Nilsberth et al, 2001; Chong et al, 2003; Barghorn et al, 2005; Wogulis et al, 2005; Hepler et al, 2006; Haass and Selkoe, 2007).

A β-Amyloid fibrils, on the other hand, are found in the amyloid plaques as large insoluble macromolecular assemblies characterized by a ‘cross-β’ fiber-like architecture. Mature fibrils are resistant to proteolytic cleavage, are unaffected by denaturant concentrations that unfold globular proteins and possess high thermostability (Booth et al, 1997; O’Nuallain et al, 2005). Amyloid fibrils from different proteins are biologically inert, although cytotoxicity appears primarily caused by soluble prefibrillar oligomers (Bucciantini...
et al., 2002). Taken together, these observations suggest an archetypal model for amyloid-associated pathologies in which disease is caused by transient toxic aggregates that eventually convert to inert amyloid deposits. These deposits would then be simple remnants of the aggregation process playing no significant role in the disease process (Lomas et al., 1992). On the other hand, amyloid fibrils are not strictly irreversible but rather in a slow dynamic equilibrium with soluble peptide (Carulla et al., 2005; O’Nuallain et al., 2005). For instance, amyloid fibrils from the PI3 kinase-SH3 domain recycle about half of their molecules over a period of weeks (Carulla et al., 2005). Inspired by the fact that disturbed lipid metabolism is increasingly considered as an important factor in AD pathogenesis, we have investigated the influence of biological lipids on the stability of amyloid fibrils of the Alzheimer’s β-peptide 1–42 (Aβ42) in relation to neurodegeneration. We focused on sphingolipids (SM) and gangliosides (GM), which are associated with amyloid deposits (Kakio et al., 2002; Devanathan et al., 2006). In addition, lipid raft domains containing cholesterol (CH), SM and GM promote Aβ aggregation and oligomer formation (Kakio et al., 2002; Yip et al., 2002; Zou et al., 2003; Gellermann et al., 2005; Kim et al., 2006). Peroxidized lipids and their derivatives such as 4-hydroxynonenal are involved in amyloid aggregation linking oxidative stress to Aβ deposition (Irminger-Finger et al., 1999). Finally, phospholipids stabilize toxic oligomers generated from monomeric Aβ (Johansson et al., 2007).

Results

**Lipids convert inert amyloid fibrils to a highly toxic species**

We measured the effect of lipids on mature amyloid fibrils of Aβ42, in terms of their toxicity to primary neurons *in vitro*. We incubated Aβ42 for 2 weeks at 25°C, at 1 mg ml⁻¹ in 50 mM Tris-HCl, pH 7.4, and 5 mM EDTA and controlled the presence of Aβ42 fibrils by electron microscopy. These mature Aβ42 fibrils were subsequently harvested by centrifugation and added to primary hippocampal mouse neurons

Figure 1 Lipid-derived Aβ protofibrils cause neurotoxicity and cell death in primary hippocampal neurons. Inert Aβ fibrils (250 mg ml⁻¹, 50 μM) were incubated with DOPC liposomes (2.5 mg ml⁻¹) overnight while shaking and subsequently added in a 1:10 dilution (5 μM and 0.25 mg ml⁻¹ final concentrations of fibrils and lipids, respectively) to primary cultures of hippocampal neurons cultured for 18 DIV (days in vitro). (A) Neutral red incorporation by neurons after treatment with lipids (0.25 mg ml⁻¹ final concentration), Aβ fibrils (5 μM final concentration) or lipid/Aβ fibrils during 48 h was assayed. DOPC liposomes alone (black), Aβ fibrils alone (green), Aβ fibril/DOPC (red), Aβ fibril/DOPC soluble fraction obtained by extensive centrifugation (blue) or resuspended Aβ fibril/DOPC insoluble pelleted fraction (yellow) are shown. Values are % of control ± s.e.m., P < 0.002, of three independent experiments performed in triplicate. (B) Neutral red incorporation by neurons treated for 24 h with various lipid mixtures (black) or with soluble fraction from Aβ fibril/lipid mixtures (white) is shown (concentrations as above). DOPC, dioleyl phosphatidylcholine; DMPG, dioleyl phosphatidylglycerol, GM1, ganglioside; SM, sphingomyelin (SM); BTE, brain total extract. Values are % of control ± s.e.m., P < 0.006, of three independent experiments performed in triplicate. Aβ fibrils (5 μM final concentration) or lipid mixtures (0.25 mg ml⁻¹ final concentration) alone had no effect on neutral red incorporation. (C) Annexin V/propidium iodide (PI) staining of primary neurons. Fluorescence microscopy images of Annexin V (green) and PI (red) staining of hippocampal neurons cultured for 18 DIV treated with GM1, SM and BTE liposomes and the soluble fraction of the same Aβ fibrils/lipid mixtures in the concentrations mentioned above are shown. Incubation with 5 μM of Aβ fibrils alone did not affect this staining (not shown). (D) Cleaved caspase-3 staining. Fluorescence microscopy images of cleaved caspase-3 staining of hippocampal neurons cultured for 18 DIV treated with SM, GM1 and BTE liposomes and soluble fractions of Aβ fibrils/lipid mixtures as mentioned above are shown. Incubation with 5 μM of Aβ fibrils alone did not affect this staining (not shown). Results reveal apoptotic cell death induced by soluble fractions of Aβ fibrils/lipid mixtures but not by Aβ fibrils or lipids alone.
Table 1 Comparison of the toxicity of SEC fractions of Aβ42 mixed with various lipids

| Lipid  | Fraction | Neutral red | A11 binding |
|--------|----------|-------------|-------------|
| DMPG   | 16.9     | 53.0±6.2    | ++          |
| Cholesterol | 16.9     | 50.5±3.5    | ++          |
| SM     | 16.9     | 43.1±2.4    | ++          |
| GM1    | 16.9     | 36.1±2.9    | ++          |
| BTE    | 16.9     | 25.2±4.3    | ++          |

Inert Aβ fibrils (250 mg ml\(^{-1}\), 50 μM) were incubated with liposomes enriched in different lipids (left column in the table) (2.5 mg ml\(^{-1}\)) overnight while shaking and then centrifuged as indicated previously. The SEC fractionation of the soluble part of Aβ42 fibril/lipid mixtures shows a peak at 16.9 ml. Toxicity of the 16.9 ml peak from the various Aβ42 fibril/lipid emulsions is quantified by neutral red incorporation into hippocampal neuronal cells. A qualitative indication of binding to the oligomer-specific A11 antibody to the 16.9 ml peak from the various Aβ42 fibril/lipid emulsions detected by dot blot is also given.

Insoluble fraction containing fractured and highly intertwined amyloid material surrounded by short amyloid fragments (Figure 2B), whereas the supernatant contained protofibrillar structures (Figure 2C), confirming fibril destabilization and resolubilization in the presence of lipids. Confocal microscopy using immunostaining with the antibody A11 that is specific for ‘soluble prefibrillar oligomers’ (Kayed et al, 2003) shows not only a granular decoration of material on the plasma membrane of primary neurons, but also significant internalization (Figure 2D) matching the behavior of prefibrillar toxic material extracted from AD brains (Chromy et al, 2003). Amyloid–lipid emulsions were further deposited under a sucrose gradient and centrifuged at 100 000 g for 1 h. We used the Aβ-specific mAb 6E10 and the oligomer-specific pAb A11 to detect Aβ species. Although both the top of the gradient and the pellet reacted with 6E10, only the top of the gradient reacted with A11 (Figure 2E), demonstrating that fibrils are indeed resolubilized and that the soluble fraction migrates in the same fraction as the liposomes, whereas insoluble amyloid material was pelleted.

Dynamic light scattering (DLS) at a detection angle of 90° relative to the incident beam detected hydrodynamic radii between 10 and 100 μm in samples of mature fibrils (Figure 3A1). When lipids were added to the sample (Figure 3A2), the hydrodynamic radius dropped to a range between 100 nm and 1 μm, indicating significant heterogeneity. A sample of lipids alone was monodispersed with an apparent radius of roughly 200 nm (Figure 3A3). A similar size distribution is observed from light scattering measured at 173° (back scattering), excluding misinterpretations due to the angular dependence of light scattering (data not shown). Both the size distribution and heterogeneity observed by light scattering are in excellent agreement with sizes observed by electron microscopy, where flexible protofibrils are observed to curl into spheroid shapes with dimensions between 100 and 300 nm (Figure 2C). Further confirmation that the amyloid fibrils revert to a protofibrillar state (Walsh et al, 1997; Hartley et al, 1999) was obtained from spectroscopic analysis, which showed intermolecular β or cross-β structure similar to that of mature amyloid fibrils (Figure 3B and C). Circular dichroism (CD) revealed an increase in the amplitude around 220 nm, but no significant shape change compared to the amyloid far UV spectrum, indicating an increase in soluble material in the amyloid–lipid mixtures with a similar β-sheet content as the amyloid fibrils (Figure 3B). Fourier-transform infrared (FTIR) spectra indicated that lipid-induced protofibrils possess a similar intermolecular β-extended structure as mature fibrils (corresponding to the spectral band at 1623 cm\(^{-1}\)), but the difference FTIR spectrum revealed some degree of unfolding in the protofibrils as compared to the mature amyloid fibrils, as was apparent from the 1647 cm\(^{-1}\) band (Figure 3C). We proceeded to analyze our lipid-induced protofibrils by size exclusion chromatography (SEC). When the supernatant of a lipid/fibril mixture was injected on an S75/HR10 column, a single peak at 15.8 ml was eluted (Figure 3D, green line), which immunostained with both the 6E10 and A11 antibodies (Figure 3D). Size determination from the elution volume yields an apparent molecular weight of approximately 9 kDa (meric Aβ).

Lipids cause mature amyloid fibrils to disassemble into a protofibrillar species

To determine the mechanism by which lipids cause the conversion of inert amyloid fibrils into a toxic state, we proceeded to the biophysical characterization of fibril–lipid mixtures. As is clear from the introduction, the atypical behavior of Aβ42 in a number of biophysical techniques has led to conflicting interpretations about the nature of the toxic species (Hepler et al, 2006). For this reason, we combine a range of biophysical assays, to obtain a maximum of information on the nature of the toxic species we here obtained. Transmission electron microscopy revealed that amyloid fibrils (Figure 2A) were converted by lipids to an
by TEM again clearly shows a heterogeneous mixture of protofibrillar oligomers with a size of 100–200 nm. To characterize the size distribution of the lipid/fibril mixture, we instead utilized an 18-angles static light scattering (SLS) detector inline with the SEC column, which allows to infer size information directly from the angular dependence of the scattered light intensity in an absolute manner that is independent from shape or gel matrix interactions. SLS indicates a strong nonlinear angle dependence in the light scattering intensity (Figure 3E), consistent with objects larger than 100 nm, and calculated molecular weights of 80–500 kDa (between 20 and 90 monomeric units). The fact that a heterogeneous sample elutes as a focused peak is consistent with strong interactions with the gel matrix, as under these conditions the elution profile is no longer determined by the size but by the strength of the column interactions and no size separation is achieved. Taken together, the methods used here are in agreement with earlier analysis of the structure (Walsh et al., 1999; Hepler et al., 2006) and toxicity (Walsh et al., 1997, 2002b) of protofibrils and indicate that the sample cannot be defined by a single molecular mass.

'Backward' protofibrils obtained by destabilizing mature fibrils using lipids are identical to 'forward' protofibrils obtained in ageing solutions of monomeric Aβ

We proceeded to compare fibril-derived protofibrils (further termed 'backward' protofibrils) with the extensively studied soluble protofibrils formed during the aggregation process of fresh Aβ (and further termed 'forward' protofibrils). When we incubated monomeric Aβ42 at 1 mg ml⁻¹ in 50 mM Tris-HCl, pH 7.4, and 1 mM EDTA at 25°C, we found, consistent with previous observations (Walsh et al., 1999; Bitan and Teplow, 2004; Klyubin et al., 2005), that neurotoxicity of forward protofibrils peaked between 24 and 48 h (Figure 1F), at which point the sample contains a mixture of protofibrils and some mature fibrils (Figure 4A). Subsequently, the sample becomes inert and highly enriched of mature amyloid fibrils (Figure 2A shows 2-week-old samples) and does not contain detectable amounts of soluble protofibrils anymore. Interestingly, toxicity of forward protofibrils and lipid-induced backward protofibrils is very similar (Figure 4B). Soluble fractions of forward protofibrils...
Figure 3  Biophysical characterization of lipid-induced protofibrils. (A) DLS spectra of mature Aβ42 fibrils (A1), fibril/lipid mixtures (A2) and pure liposome preparations (A3, DOPC). Apparent hydrodynamic radii are indicated on the x axis (logarithmic scale), the y axis shows the evolution of the signal during the experiment (20 s) and the color code indicates a relative intensity scale between 0 and 1. The liposome spectrum (A3, 2.5 mg ml⁻¹ lipid concentration) reveals strong monodispersity in the sample with an average hydrodynamic radius just above 100 nm, whereas the amyloid fibril sample (A1, 50 μM peptide concentration) contains a wider range of molecular sizes, ranging from 5 to 100 μm approximately. The spectrum of the amyloid–lipid mixture (A2, 50 μM peptide concentration and 2.5 mg ml⁻¹ lipid concentration) shows a complete loss of signal for hydrodynamic radii above 5 μm. The majority of the signal shows strong heterogeneity with sizes ranging from 100 nm and 1 μm, consistent with the disassembly of amyloid fibrils into smaller species. (B) Far UV CD spectroscopy gives information on the secondary structure of material in solution. The spectra of Aβ42 amyloid fibrils in isolation (50 μM) and in the presence of liposomes (2.5 mg ml⁻¹) containing DMPG display a marked increase in the intensity of the spectrum around 220 nm, while the overall shape of the spectrum is constant. This is consistent with an increase in the amount of soluble material rich in β-sheet content. Similar results were obtained with other lipids (not shown). (C) FTIR yields information on the secondary structure of both the soluble and insoluble material in the sample. The spectra of mature Aβ42 fibrils (50 μM) and fibril/lipid mixtures (BTE, 2.5 mg ml⁻¹) show a strong cross-β signal at 1623 cm⁻¹, consistent with a comparable β-sheet content for amyloid fibrils and protofibrils. However, the difference spectrum reveals an additional band at 1647 cm⁻¹, corresponding to the formation of random coil in the amyloid/lipid mixture, consistent with partial fibril disassembly. (D) SEC on a GEHealthcare S75/HR10 column of ‘forward’ (blue line) and lipid-induced ‘backward’ oligomers (green line). For each sample, 200 μl of an amyloid–lipid mixture containing 250 μg of Aβ42 peptide and 2.5 mg ml⁻¹ lipid were injected. Notice peak elutions at 15.8 ml and 16.9 ml, respectively. Upon 0.1% SDS treatment, backward oligomers elute at the same position as forward oligomers, whereas lipids elute in the void volume (black line). For comparison, an elution trace for monomeric Aβ42 is also shown (red line, elution peak at 21 ml). Elution profiles of amyloid fibril samples show no noticeable peaks. Inset show immunostaining of the lipid fraction and the Aβ42/lipid-induced oligomer fraction of the black profile using the antibody A11, which specifically detects oligomers, and the 6E10 mAb, which is specific for Aβ. (E) Eighteen-angles SLS, placed inline with the SEC system, allows to determine molecular mass in an absolute manner that does not depend on interactions with column matrix (as is the case in SEC) or assumed molecular shape (as is the case in DLS). The 15.8 ml peak shown in Figure 3D was analyzed in this manner. The Zimm plot (see Materials and methods) in Figure 3E shows the clear nonlinear dependence of the light scattering intensity with scattering angle, consistent with a radius of gyration larger than 80 nm. The red curve indicates a fit to a quadratic equation (R-squared value >0.9). The molecular masses obtained in this manner vary throughout the peak, in agreement with the heterogeneity observed in DLS and electron microscopy and consistent with a total loss of size-sorting effects from the column due to nonspecific matrix interactions. Masses obtained vary from 80 to 500 kDa throughout the peak.
Morphology and toxicity of forward protofibrils. (A) Electron micrograph of 48 h aged solution of freshly dissolved Aβ42 reveals a mixture of protofibrillar intermediates and the first appearance of some amyloid fibrils. (B) Neutral red incorporation by hippocampal neurons generated from monomers during 48 h aggregation (5 μM) and soluble fractions of Aβ fibrils/lipid mixtures at 5 μM and 0.25 mg ml⁻¹ final concentrations of fibrils and lipids, respectively (termed as ‘backward lipid-derived protofibrils’, see text). Values are % of control ± s.e.m., *P<0.008, of two independent experiments performed in quadruplicates.

In vivo toxicity of backward protofibrils in the brain of adult mice

To further characterize the physiological relevance of the lipid-induced ‘backward’ oligomers, we proceeded to bilateral intraventricular injection (3 μl) of mature Aβ42 amyloid fibrils, lipids and supernatants from amyloid–lipid mixtures into the brain of adult mice. Immunostaining of brain samples with the 6E10 antibody demonstrated the effective delivery of Aβ in the ventricles when both fibrils and backward oligomers were injected into the animals (Figure 5A). However, within 90 min of injection, we also observed Aβ42 immunoreactivity away from the needle tracks into the cortex (Figure 5A), with oligomeric preparations but not with Aβ fibrils. With the latter, staining remained mostly associated with the ventricular walls (Figure 5A). When we analyzed brain areas further away from the needle track, we observed very little immunostaining after Aβ fibril injections, whereas backward oligomers caused significant staining of the hippocampal areas (Figure 5B), indicating rapid diffusion of (some of) these species into the brain parenchyma (Figure 5B). Interestingly, the single injection with backward oligomers caused also mild neurotoxicity restricted to the area of the frontal cortex (but not hippocampus) that was exposed to the highest concentrations of toxic oligomers as revealed by immunostaining for cleaved caspase 3 (Figure 5C) and phosphorylated tau (Figure 5D). Lipid or mature fibrils injection alone did not cause toxicity in the frontal cortex (Figure 5C and D).

Backward and forward protofibrils cause similar memory defects in mouse

To further assess if the lipid-induced ‘backward’ protofibrils might cause functional deficits relevant to AD, we evaluated the acute biological effects of the injected backward oligomers in exploratory and memory/learning tests. Open-field recording revealed increased path length covered, higher velocity and increased frequencies of center visits, but declined time spent in the center by animals injected with backward oligomers, in sharp contrast to animals injected with lipid samples or mature fibrils alone. As all animals were treated identically, the backward oligomers appear to specifically affect brain functions that cause hyperlocomotion and hyperactivation (Figure 6A). In a light–dark step through task (passive avoidance test), animals were allowed to memorize the electrical shock that follows entrance to a dark compartment. When the test was repeated 24 h later, animals injected with lipids or mature fibrils correctly recalled the electroshock and avoided to enter the dark room. Injection of backward oligomers before the shock event prevented animals from successful memory formation, as was evidenced by uninhibited entering of the dark compartment 24 h after electroshock (Figure 6B). In addition, contextual and auditory-cue fear conditioning showed that injection of backward oligomers 90 min before conditioning severely disturbed typical freezing behavior 24 h later when the animals were exposed again to the same contextual or auditory stimulus, in contrast to the control mice injected with lipids or mature amyloid alone (Figure 6C). One week after oligomer injection, mice appeared to have recovered completely and could no longer be distinguished in open-field activity from control or untreated animals (not shown in figure). Thus, a single injection of lipid-induced backward oligomers appears not to have caused persistent functional defects, which is in excellent agreement with earlier studies of ‘forward’ oligomers, which were reported to have immediate but transient effects on synaptic function (Wang and Hecht, 2002; Chromy et al.,
Therefore, to directly compare the behavioral effects of forward and backwards protofibrils, we performed identical behavioral tests on animals injected with forward oligomers generated from Aβ42 monomers that were incubated for 48 h at room temperature at a concentration of 1 mg ml⁻¹. These animals showed very similar behavioral defects: open-field recording indicated hyperlocomotion and hyperactivation effects (result not shown) and light–dark step through tests (passive avoidance) showed that animals injected with forward oligomers failed to memorize the electrical shock, in contrast to control individuals injected with buffer (Figure 6D). In addition, contextual and auditory-cue fear conditioning showed that injection of forward oligomers 90 min before conditioning severely disturbed freezing behavior 24 h later, in contrast to the control mice (Figure 6E).

Our results confirm the behavioral effects of forward protofibrils generated from Aβ monomers (Hartley et al., 1999; Walsh et al., 2002a; Kamenetz et al., 2003; Cleary et al., 2005; Klyubin et al., 2005; Lesne et al., 2006; Townsend et al., 2006) and show that lipid-induced protofibrils generated from mature Aβ fibrils have very similar pathophysiological effects.

Discussion

We demonstrate in the current work that amyloid fibrils, usually considered as highly stable and biologically inert structures, can be destabilized and easily reverted to soluble and highly toxic Aβ aggregates by biological lipids that are present in the brain. This suggests that part of the critical
balance between toxic and inert Aβ pools is determined by the relative amounts of lipids in the direct environment of the plaques. Remarkably, the toxic species we identify shares many properties with previously characterized ‘forward’ oligomeric aggregates in terms of biophysical, cell biological and behavioral assays. Although in SEC and other biophysical assays the impression could arise that these structures are homogenous, further extensive biophysical characterization indicates that the size distribution of these oligomeric aggregates is rather heterogeneous in nature, ranging from 80 to 500 kDa, although their morphology is protofibrillar. The appearance of these species indeed varies somewhat with the assay used, likely reflecting their dynamic nature. In that regard, our findings are very similar to the overall picture emerging from the literature and synthesized recently by Hepler et al (2006) that also the classical forward oligomeric Aβ structures have to be considered as a spectrum of dynamic structures that are likely in fast equilibrium with each other. Lipids are apparently promoting the equilibrium toward the protofibrillar pools, inducing toxicity of the amyloid mixture. Interestingly our data also confirm that these oligomeric structures diffuse very rapidly throughout the brain and preferentially localize to specific regions of the brain, such as the hippocampus.

Our findings have important implications for the understanding and the treatment of amyloidoses and, in particular, AD. They suggest the possibility that inert amyloid plaques or fibrils could be turned into highly toxic oligomers when local physicochemical parameters are altered due, for instance, to a change in lipid metabolism. Our results could explain why the amount of amyloid deposits and the severity of associated disease symptoms in AD do not necessarily correlate. Individual and temporal differences in brain lipid content could indeed explain why some patients with large amounts...
of amyloid deposits display little symptomatic disease, although others are severely affected. In any event, our data strongly suggest that amyloid plaques, although apparently biologically inert, should not be considered as inert remnants of the aggregation process, as the amyloid fibrils they contain can, under certain conditions, be rapidly reverted to toxic species. In that sense, the amyloid plaques should rather be considered as reservoirs of toxicity.

Materials and methods

Chemicals
Alzheimer’s β-peptide 1–42 was purchased from Sigma-Aldrich. All purified and synthetic lipids were obtained from Avanti Lipids (USA). Uraanyl acetate was obtained from BDH.

Preparation of lipid vesicles and liposomes
All lipids were obtained from Avanti Polar Lipids (USA) except the ganglioside GM1, which was obtained from Larodan Chemicals (Sweden). The stock concentration was 20 mg ml\(^{-1}\). Cholesterol or GM1. Therefore, we used a more complex composition as follows: (i) pure DOPC; (ii) 50% DOPC, 50% DMPG; (iii) 30% DOPC, 30% DMPG, 30% cholesterol; (iv) 30% DOPC, 30% DMPG, 30% cholesterol, 10% GM1; (v) 30% DOPC, 30% DMPG, 30% cholesterol, 10% SM; (vi) 30% DOPC, 30% DMPG, 30% cholesterol, 10% brain total extract.

Preparation of amyloid fibrils, amyloid/lipid mixtures and A\(\beta\) oligomers
Amyloid fibrils of the Alzheimer’s β-peptide 1–42 were obtained by incubation of 200μM peptide solution in 50 mM Tris, pH 7.5, for 2 weeks at room temperature. Amyloid fibril/lipid mixtures were prepared by diluting fibril and lipidosome stock solutions in liposome buffer and incubating for 1–16h at room temperature, shaking at 700 c.p.m. Oligomers were generated from monomers by incubation of 200 μM peptide solution in 50 mM Tris, pH 7.5, for 48 h and subsequent extensive centrifugation, and the supernatant as the oligomer-enriched preparation was used immediately. The presence of oligomers in the supernatant was validated by probing with anti-oligomer-specific antibody (A11).

Additional methods can be found in Supplementary data.

Supplementary data
Supplementary data are available at The EMBO Journal Online (http://www.embojournal.org).

Acknowledgements
ICM was supported by a PhD scholarship from the Boehringer Ingelheim Fonds (BIF, Germany), Foundation for Basic Research in Biomedicine, and subsequently a PhD scholarship from the Fonds voor Scientiﬁsch Onderzoek (Viborg, VIB, Denmark). A Switch laboratory was supported by a grant from the Federal Office for Scientiﬁc Affairs, Belgium (IUAP P6/43), and the Fund for Scientiﬁc Research, Flanders.

References
Aksenov MY, Aksenova MV, Butterfield DA, Hensley K, Vigo-Pelfrey C, Carney JM (1996) Glutamine synthetase-induced enhancement of beta-amyloid peptide A beta (1–40) neurotoxicity accompanied by aggregation of fibril formation and A beta fragmentation. J Neurochem 66: 2050–2056
Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bittner RS, Harlan J, Barlow E, Ebert U, Hillen H (2005) Globular amyloid beta-peptide oligomer—a homogenous and stable neuropathological protein in Alzheimer’s disease. J Neurochem 95: 834–847
Bian G, Teplow DB (2004) Rapid photochemical cross-linking—a new tool for studies of metastable, amyloidogenic protein assemblies. Acc Chem Res 37: 357–364
Bian G, Voller S, Teplow DB (2003) Elucidation of primary structure elements controlling early amyloid beta-protein oligomerization. J Biol Chem 278: 34882–34889
Booth DR, Sunde M, Bellotti V, Robinson CV, Hutchinson WL, Fraser PE, Hawkins PN, Dobson CM, Radford SE, Blake CC, Pepys MB (1997) Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. Nature 385: 787–793
Bucciantini M, Giannoni E, Chiti F, Baroni F, Formilli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416: 507–511
Carulla N, Caddy GL, Hall DR, Zurdo J, Gairi M, Feliz M, Giralt E, Robinson CV, Dobson CM (2005) Molecular recycling within the fibril. J Biol Chem 280: 690–698
Chromy BA, Nowak RJ, Lambert MP, Viola KL, Chang L, Velasco PT, Jones BW, Fernandez SJ, Lacor PN, Horowitz P, Finch CE, Kraft GA, Klein WL (2003) Self-assembly of Aβ (1–42) into globular neurotoxins. Biochemistry 42: 12749–12760
Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005) Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat Neurosci 8: 79–84
Devanathan S, Salamon Z, Lindblom G, Grobner G, Tollin G (2006) Effects of sphingomyelin, cholesterol and zinc ions on the binding, insertion and aggregation of the amyloid Aβ(1–40) peptide in solid-supported lipid bilayers. FEBS J 273: 1389–1402
Games D, Adams A, D’Alessandri R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberberg I, Little S, Masliha E, McConlogue L, et al (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V171F beta-amyloid precursor protein. Nature 373: 523–527
Gellerman GP, Appel TR, Tannert A, Radestock A, Hortschansky P, Schroeck F, Leisner C, Lutkepohl T, Schrötzer M, Rocken C, Pras M, Linke RP, Dierick S, van Duijnhoven M (2005) Raft lipids as common components of human extracellular amyloid fibrils. Proc Natl Acad Sci USA 102: 6297–6302
Glabe CG, Kayed R (2006) Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. Neuronology 66: 574–578
Gong Y, Chang L, Viola KL, Lacor PN, Lamberts MP, Finch CE, Kraft GA, Klein WL (2005) Alzheimer’s disease-affected brain: presence of oligomeric A beta lipids (ADDLs) suggests a molecular
basis for reversible memory loss. *Proc Natl Acad Sci USA* **100**: 10417–10422

Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer’s amyloid beta-peptide. *Nat Rev Mol Cell Biol* **8**: 101–112

Hardy J (2002) Testing times for the ‘amyloid cascade hypothesis’. *Neurobiol Aging* **23**: 1073–1074

Hardy JA, Higgins GA (1992) Alzheimer’s disease: the amyloid cascade hypothesis. *Science* **256**: 184–185

Harper JD, Wong SS, Lieber CM, Lansbury PT (1997) Observation of metastable Abeta amyloid protofibrils by atomic force microscopy. *Chem Biol* **4**: 119–125

Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, Teplow DB, Selkoe DJ (1999) Protofibrillar intermediates of amyloid-beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J Neurosci* **19**: 8876–8884

Hepler RW, Grimm KM, Nahas DD, Breese R, Dodson EC, Acton P, Keller PM, Vagner M, Wang H, Shughrepe P, Kinney G, Joyce JG (2006) Solution state characterization of amyloid beta-derived diffusible ligands. *Biochemistry* **45**: 15157–15167

IrmingerFinger I, Siegel BD, Leung WC (1999) The functions of breast cancer susceptibility gene 1 (BRCA1) product and its associated proteins. *J Biol Chem* **274**: 117–128

Johansson A-S, Garlind A, Berglind-Dehlin F, Karlsson G, Edwards K, Gellerfors P, Ekhholm-Pettersson F, Palmblad J, Lannfelt L (2007) Docosahexaenoic acid stabilizes soluble amyloid-beta protofibrils and sustains amyloid-beta-induced neurotoxicity in vitro. *FEBS J* **274**: 990–1000

Kakio A, Nishimoto S, Yanagisawa K, Kozutsumi Y, Matsuzaki K (2002) Interactions of amyloid beta-protein with various gangliosides in rat-like membranes: importance of GM1 ganglioside-bound form as an endogenous seed for Alzheimer amyloid. *Biochemistry* **41**: 7385–7390

Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**: 486–489

Kim SI, Yi JS, Ko YC (2006) Amyloid beta oligomerization is induced by brain lipid rafts. *J Cell Biochem* **99**: 878–889

Klyubin I, Walsh DM, Lemere CA, Cullen WK, Shankar GM, Betts V, Spooner ET, Jang L, Anwyl R, Selkoe DJ, Rowan MJ (2005) Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo. *Nat Med* **11**: 556–561

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Tommer B, Viola KL, Wals P, Zhang C, Finch CE, Kraft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA* **95**: 6448–6453

Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury Jr PT (2002) Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature* **418**: 291

Lesne S, Koh MT, Kotilinne L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* **440**: 352–357

Lomas DA, Evans DL, Finch JT, Carrell RW (1992) The mechanism of Z alpha 1-antitrypsin accumulation in the liver. *Nature* **357**: 605–607

McLean CA, Cherry RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL (1999) Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer’s disease. *Ann Neurol* **46**: 860–866

Nilsberth C, Westlund-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, Stenh C, Luthman J, Teplow DB, Younkin SG, Naslund J, Lannfelt L (2001) The ‘Arctic’ APP mutation (E693G) causes Alzheimer’s disease by enhanced Abeta protofibril formation. *Nat Neurosci* **4**: 887–893

O’Nuallain B, Shiri-prasad S, Kheretal I, Wetzal R (2005) Thermodynamics of A beta(1–40) amyloid fibril elongation. *Biochemistry* **44**: 12709–12718

Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB, Selkoe DJ (1995) Aggregation of secreted amyloid beta-protein into sodium dodecyl sulfate-stable oligomers in cell culture. *J Biol Chem* **270**: 9564–9570

Podlisny MB, Walsh DM, Amarante P, Ostaszewski BL, Stimson ER, Maggio JE, Teplow DB, Selkoe DJ (1998) Oligomerization of endogenous and synthetic amyloid beta-protein at nanomolar levels in cell culture and stabilization of monomer by Congo red. *Biochemistry* **37**: 5602–3611

Price JL, Morris JC (1999) Tangles and plaques in nondemented aging and ‘preclinical’ Alzheimer’s disease. *Ann Neurol* **45**: 358–368

Selkoe DJ (1991) The molecular pathology of Alzheimer’s disease. *Neuron* **6**: 487–498

Terry RD (2001) Commentary. Copernicus revisited: amyloid beta in Alzheimer disease. *Neurobiol Aging* **22**: 155 discussion 161–153

Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006) Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimerers. *J Physiol* **572**: 477–492

Walsh DM, Hartley DM, Kusumoto Y, Fezou Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem* **274**: 25945–25952

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002a) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**: 535–539

Walsh DM, Klyubin I, Fadeeva JV, Rowan MJ, Selkoe DJ (2002b) Amyloid-beta oligomers: their production, toxicity and therapeutic inhibition. *Biochem Soc Trans* **30**: 552–557

Walsh DM, Lomakin A, Benedek GB, Condron MM, Teplow DB (1997) Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *J Biol Chem* **272**: 22364–22372

Wang W, Hecht MH (2002) Rationally designed mutations convert de novo amyloid-like fibrils into monomeric beta-sheet proteins. *Proc Natl Acad Sci USA* **99**: 2760–2765

Wogulis M, Wright S, Cunningham D, Chilcote T, Powell K, Rydel RE (2005) Nucleation-dependent polymerization is an essential component of amyloid-mediated neuronal cell death. *J Neurosci* **25**: 1071–1080

Yip CM, Darabie AA, McLaurin J (2002) Abeta42-peptide assembly on lipid bilayers. *J Mol Biol* **318**: 97–107

Zou K, Kim D, Kakio A, Byun K, Gong JS, Kim J, Kim M, Sawamura N, Nishimoto S, Matsuzaki K, Lee Y, Yanagisawa K, Michikawa M (2003) Amyloid beta-protein (Abeta)1-40 protects neurons from damage induced by Abeta1-42 in culture and in rat brain. *J Neurochem* **87**: 609–619

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