

## LETTERS

# A specific amyloid- $\beta$ protein assembly in the brain impairs memory

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Memory function often declines with age<sup>1</sup>, and is believed to deteriorate initially because of changes in synaptic function rather than loss of neurons<sup>2</sup>. Some individuals then go on to develop Alzheimer's disease with neurodegeneration. Here we use *Tg2576* mice, which express a human amyloid- $\beta$  precursor protein (APP) variant linked to Alzheimer's disease, to investigate the cause of memory decline in the absence of neurodegeneration or amyloid- $\beta$  protein amyloidosis. Young *Tg2576* mice (<6 months old) have normal memory and lack neuropathology, middle-aged mice (6–14 months old) develop memory deficits without neuronal loss, and old mice (>14 months old) form abundant neuritic plaques containing amyloid- $\beta$  (refs 3–6). We found that memory deficits in middle-aged *Tg2576* mice are caused by the extracellular accumulation of a 56-kDa soluble amyloid- $\beta$  assembly, which we term A $\beta$ \*56 (A $\beta$  star 56). A $\beta$ \*56 purified from the brains of impaired *Tg2576* mice disrupts memory when administered to young rats. We propose that A $\beta$ \*56 impairs memory independently of plaques or neuronal loss, and may contribute to cognitive deficits associated with Alzheimer's disease.

Poor memory function can predict Alzheimer's disease up to 15 years before diagnosis<sup>3</sup>, and non-demented individuals at risk genetically for Alzheimer's disease show abnormalities in functional brain imaging tests<sup>8,9</sup>. These and other studies imply that Alzheimer's disease has an insidious onset, which blurs the boundary between age-associated memory impairment and Alzheimer's disease<sup>10</sup>. *Tg(APP<sup>SWE</sup>)2576Kahs* mice (hereafter *Tg2576* mice) express a human amyloid- $\beta$  precursor protein (APP) variant linked to Alzheimer's disease, and develop many neuropathological features of Alzheimer's, including amyloid plaques, dystrophic neurites and inflammatory changes<sup>3,4</sup>. However, *Tg2576* mice lack neurofibrillary tangles, significant neuronal loss and gross atrophy<sup>4</sup>. They may therefore be a good model to study pre-clinical stages of Alzheimer's disease before the diagnosis of dementia or the onset of neuronal loss.

In *Tg2576* mice, as in other APP transgenic mice, there is strong evidence that amyloid- $\beta$  (A $\beta$ ) is responsible for age-related memory decline<sup>6,12,13</sup>. However, there are several paradoxical findings about the relationship between A $\beta$  and cognitive decline that suggest a complex role for A $\beta$  in cognitive impairment. For example, spatial reference memory in *Tg2576* mice declines modestly but significantly at 6 months of age and then remains stable for 7 to 8 months (Fig. 1a, b). However, no candidate A $\beta$  species measured to date corresponds with the decline in memory observed at 6 months and the cognitive stability observed thereafter (see Supplementary Table 1). Hence, we are faced with the paradox that a rapidly increasing amount of A $\beta$ , the molecule believed to be responsible

for memory loss, is associated with no change in memory function. One solution to this conundrum is to posit the existence of soluble A $\beta$  assemblies that disrupt memory<sup>6,14</sup>, which we designated A $\beta$ \* (A $\beta$  star) and sought to identify in *Tg2576* mice.

A challenge in analysing A $\beta$  in the brain lies in reliably separating the specific cellular pools of A $\beta$  (for example, extracellular, intracellular, membrane-associated and insoluble). We overcame this obstacle by developing a high-fidelity extraction procedure that separates proteins in known cellular compartments (Supplementary Fig. 1). Our new extraction method allowed us to quantify and compare four independent pools of transgene-derived A $\beta$  species.

To resolve the problem of a mismatch between A $\beta$  levels and memory deficits, we used our extraction procedure to search for A $\beta$ \* in *Tg2576* mice between 4 and 25 months of age. We required candidate A $\beta$ \* molecules to satisfy two criteria. First, their appearance should coincide with memory loss at 6 months. Second, their levels should remain stable in middle-aged mice (6–14 months old). By immunoblotting immunoglobulin-depleted forebrain extracts, we found a set of apparent assemblies of A $\beta$  in the soluble, extracellular-enriched fraction from 6-month-old mice (Fig. 1c). In addition to a faint 4-kDa band corresponding to A $\beta$  monomers, 6E10- and 4G8-immunoreactive proteins (see Methods) were detected at molecular masses theoretically corresponding to trimeric (14 kDa), hexameric (27 kDa), nonameric (40 kDa) and dodecameric (56 kDa) A $\beta$ <sub>1–42</sub> assemblies. These species represent multiples of trimeric A $\beta$  oligomers, with high-molecular-mass assemblies (>20 kDa) appearing in mice older than 6 months. The detection of similar bands using 6E10 and 4G8 antibodies excludes the possibility that they represent degradation products of soluble APP, which lacks the mid-domain A $\beta$  epitope (A $\beta$ <sub>17–24</sub>) recognized by 4G8 (Supplementary Fig. 2a). The bands were not recognized by 22C11 or APPC17-Cter antibodies, indicating they were neither APP nor APP cleavage-end products (data not shown).

Although this result suggests that ageing induces A $\beta$  trimers to associate and form high-molecular-mass assemblies, we also considered the possibility that they might represent A $\beta$  oligomers complexed to binding proteins. However, this is unlikely on the basis of their biochemical properties and immunospecificity. First, we examined their properties in urea, a common denaturant of globular proteins<sup>17</sup>. To our surprise, the A $\beta$  oligomers were unaltered in SDS–polyacrylamide gel electrophoresis (SDS–PAGE) containing 8 M urea (Fig. 2a). However, when exposed to  $\geq 10\%$  hexafluoroisopropanol (HFIP), a solvent with strong hydrogen-bonding properties, the theoretical hexamers, nonamers and dodecamers depolymerized, with a parallel increase in levels of tetramers, trimers and, to a lesser extent, monomers (Fig. 2b). In >20% HFIP, only the

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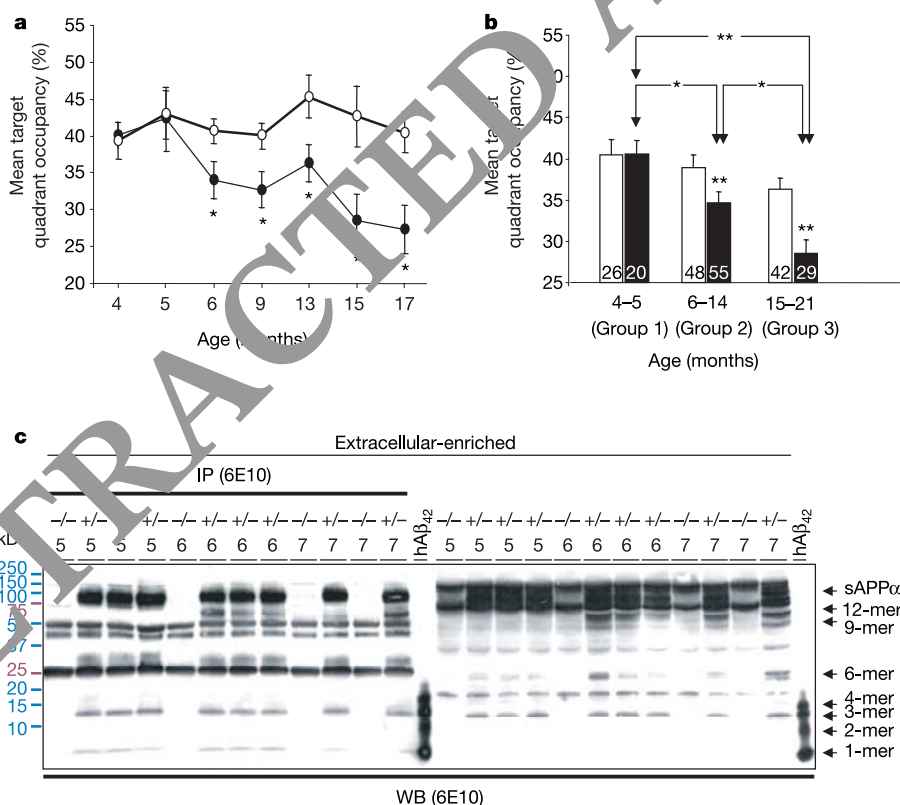
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putative trimers remained; these dissociated into A $\beta$  monomers in >55% HFIP (not shown). These data suggest that the high-molecular-mass A $\beta$  complexes are held together by urea-resistant hydrogen bonds, not by covalent bonds. In addition, the greater resistance of trimers to denaturation supports our conjecture that trimers are the fundamental A $\beta$  assembly unit *in vivo*. Second, A11 antiserum, which specifically detects soluble amyloid assemblies distinct from fibrillar A $\beta$ , detected only 27–56 kDa complexes in extracellular-enriched extracts, confirming previous observations that A11 reacts with A $\beta$  oligomers larger than tetramers<sup>18</sup> (Supplementary Fig. 2b). The immunoreactivity of the high-molecular-mass complexes with 6E10, 4G8 and A11 antibodies indicates that they are soluble A $\beta$  oligomers. Finally, we examined the native size of soluble A $\beta$  assemblies under non-denaturing conditions in order to demonstrate that they were not artificially generated during SDS–PAGE. Extracellular-enriched extracts were fractionated by non-denaturing size-exclusion chromatography (SEC) and subjected to SDS–PAGE (Fig. 2c). High- and low-molecular-mass A $\beta$  assemblies (at expected intervals) were collected in different fractions, arguing against the possibility that SDS or self-oligomerization triggered the formation of the A $\beta$  oligomers. Collectively, our results suggest that endogenous A $\beta$  forms a ladder of stable, soluble, physiological assemblies comprised of trimers and multiples of trimers in *Tg2576* mice older than 6 months.

Trimers and hexamers were excluded as components of A $\beta^*$ ,

because they were present before memory impairment (<6 months). However, a 56-kDa band appeared in extracts at 6 months of age, along with lesser quantities of a 40-kDa species; these correspond to theoretical A $\beta_{1-42}$  dodecamers and nonamers, respectively (Fig. 1c). The mean levels of the soluble A $\beta$  assemblies remained stable after 6 months of age, although there was considerable variability between animals of the same age (Supplementary Fig. 2c–e). As the 56-kDa and 40-kDa species appeared at 6 months of age and remained stable between the ages of 6 and 13 months, they fulfilled the criteria for being designated as A $\beta^*$  and thus represented viable candidates for A $\beta$  assemblies that cause memory deficits. We found no further increase in A $\beta$  assemblies in old mice to correspond to the second drop in memory function at 15 months (Fig. 1a, b); by this age it is possible that the abundant plaques with prominent dystrophic neurites disrupt synaptic function sufficient to cause further memory impairment<sup>19</sup>.

The variability in levels of A $\beta$  assemblies between animals of the same age provided an opportunity to examine correlations between the different A $\beta$  oligomers and memory impairment. To do this, we compared spatial memory and levels of soluble A $\beta$  species in two groups of 5- and 6-month-old *Tg2576* mice (Fig. 3). Monomers, trimers and hexamers of A $\beta$  did not correlate significantly with performance in 5- or 6-month-old mice (Fig. 3a–f). However, we observed significant inverse relationships between the levels of 56-kDa and 40-kDa assemblies and memory performance (Fig. 3g, h), with



**Figure 1 | Temporal patterns of soluble A $\beta$  oligomers and memory decline in *Tg2576* mice.** **a**, Memory retention in 4–17-month-old mice shows a progressive but irregular decline with periods of stability. Filled circles, *Tg2576*<sup>+/-</sup> mice; open circles, *Tg2576*<sup>-/-</sup> mice. *Tg2576*<sup>-/-</sup> and *Tg2576*<sup>+/-</sup> denote mice harboring no (non-transgenic) or one transgene array, respectively. Asterisk, ANOVA ( $P < 0.01$ ) followed by *t*-test ( $P < 0.01$ ). **b**, Temporal analysis of spatial memory shows three stages of performance. Filled bars, *Tg2576*<sup>+/-</sup> mice; open bars *Tg2576*<sup>-/-</sup>. Numbers inside bars denote numbers of mice. Asterisk,  $P < 0.01$ ; two asterisks,  $P < 0.001$  (ANOVA). Target quadrant occupancy scores ( $\pm$  s.e.m.) during probe trials are a measure of spatial reference memory retention. **c**, Identification of A $\beta$

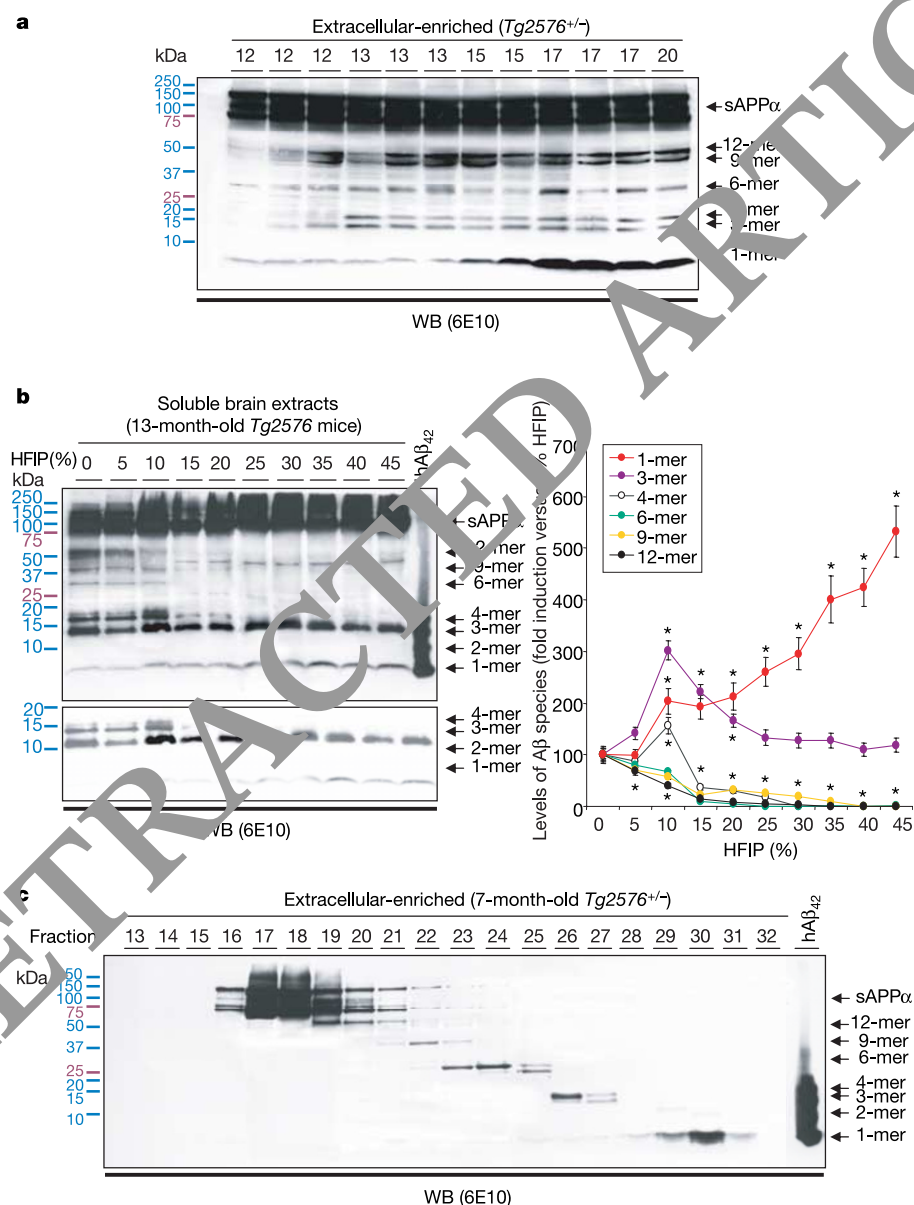
oligomers in soluble, extracellular-enriched extracts of proteins from brains of 5-, 6-, and 7-month-old mice (age indicated above lanes), assessed by western blot (WB) with or without immunoprecipitation (IP). The intensity of the 40-kDa band was  $33.92 \pm 12.5\%$  (mean  $\pm$  s.e.m.) ( $n = 4$ ) of the intensity of the 56-kDa band. Synthetic human A $\beta_{1-42}$  peptide (hA $\beta_{42}$ ) was used as a size marker and positive control (right lane). Arrows indicate respective migration positions of monomers (1-mer), dimers (2-mer), trimers (3-mer), tetramers (4-mer), hexamers (6-mer), nonamers (9-mer), dodecamers (12-mer) and sAPP $\alpha$  (secreted form of APP that has been cleaved by  $\alpha$ -secretase).

the 56-kDa assembly correlating substantially better than the 40-kDa assembly ( $r^2 = 0.66$  and  $0.45$ , respectively). There were no correlations between the levels of any A $\beta$  oligomers and performance in the cued phase of a water maze test (see Methods), arguing against their effects on non-cognitive aspects of behaviour (Supplementary Fig. 3).

Because intracellular A $\beta$  has been proposed to disrupt memory in  $3 \times$  Tg-AD transgenic mice (which express APP, presenilin-1 and tau variants)<sup>20</sup>, we performed additional analyses to evaluate the potential accumulation of A $\beta$  species within brain cells. Only trimeric and monomeric A $\beta$  species were detected in the soluble, intracellular-enriched fraction, with no modulation between 5 and 6 months of age (Supplementary Fig. 4a). We also examined full-length

APP and C-terminal fragments (CTFs) in membrane-associated fractions, and found no change in the levels of APP, CTF- $\beta$ s or CTF- $\alpha$  (Supplementary Fig. 4b, c). In cytosolic extracts of primary Tg2576 cortical neurons cultured from 14–15-day-old embryos, monomers and trimers were the only A $\beta$  species detected (Supplementary Fig. 5), supporting our *in vivo* findings in young Tg2576 mice. Thus, we found no intracellular or membrane-associated A $\beta$  species or CTFs correlating with the onset of memory deficits in 6-month-old Tg2576 mice.

The observations that the 56-kDa A $\beta$  assembly appears at 6 months of age (when memory declines), that its levels are stable in ageing mice, that it is more abundant than the 40-kDa complex (Fig. 1c), and that it shows the strongest correlation with memory



**Figure 2 | Biochemical properties of A $\beta$  assemblies in Tg2576 mice.** **a**, The presence of 8 M urea did not alter the electrophoretic pattern of A $\beta$  oligomers in extracellular-enriched extracts from 12- to 20-month-old brains of Tg2576<sup>+/-</sup> mice that were probed with 6E10 antibodies. **b**, Soluble high-molecular-mass A $\beta$  oligomers depolymerize in  $\geq 15\%$  HFIP, with concomitant enrichment of both monomeric and trimeric A $\beta$  (lower exposure provided for enhanced contrast). The data for fold-change in A $\beta$  species (right panel) is the mean  $\pm$  s.e.m. of 3 experiments. **c**, SDS-PAGE analysis of brain extracts from 7-month-old Tg2576<sup>+/-</sup> mice fractionated by

SDS-free size-exclusion chromatography (SEC) shows that A $\beta$  oligomers migrate at expected molecular masses. The data confirm that high-molecular-mass A $\beta$  oligomers are not artificially generated from monomeric or trimeric A $\beta$  species during electrophoresis, and that trimers are not degradation products of high-molecular-mass oligomers. Bands revealed at 75 and 150 kDa are non-specific bands caused by the modified avidin used for immunoblotting (also present in blots of extracts from non-transgenic mice).

impairment suggest that it is the most likely candidate for A $\beta^*$ , which we thus named A $\beta^*$ 56. However, so far our data on the relationship between A $\beta^*$ 56 and memory impairment are correlative rather than causative. To determine whether A $\beta^*$ 56 has the ability to disrupt memory function directly, we purified it from the brains of impaired Tg2576 mice, applied it to young rats and assayed its effect using the Morris water maze.

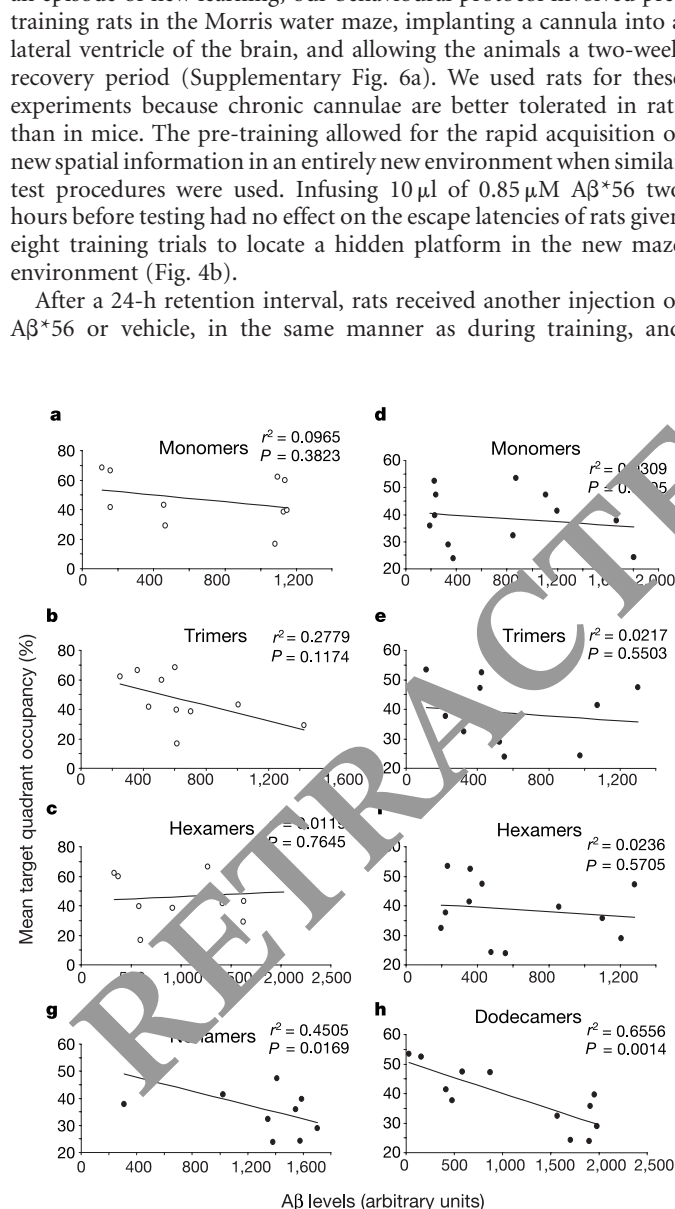
We purified A $\beta^*$ 56 using immunoaffinity chromatography (with 4G8 antibodies) followed by size-exclusion chromatography. This procedure yielded preparations of A $\beta^*$ 56 that ran as a single band on silver-stained gels (Fig. 4a), were recognized by 6E10 antibodies (Fig. 4a) and revealed A $\beta$  as a core component when analysed by liquid chromatography tandem mass spectrometry (Supplementary Fig. 7).

To allow observation of the effects of A $\beta^*$ 56 on spatial memory for an episode of new learning, our behavioural protocol involved pre-training rats in the Morris water maze, implanting a cannula into a lateral ventricle of the brain, and allowing the animals a two-week recovery period (Supplementary Fig. 6a). We used rats for these experiments because chronic cannulae are better tolerated in rats than in mice. The pre-training allowed for the rapid acquisition of new spatial information in an entirely new environment when similar test procedures were used. Infusing 10  $\mu$ l of 0.85  $\mu$ M A $\beta^*$ 56 two hours before testing had no effect on the escape latencies of rats given eight training trials to locate a hidden platform in the new maze environment (Fig. 4b).

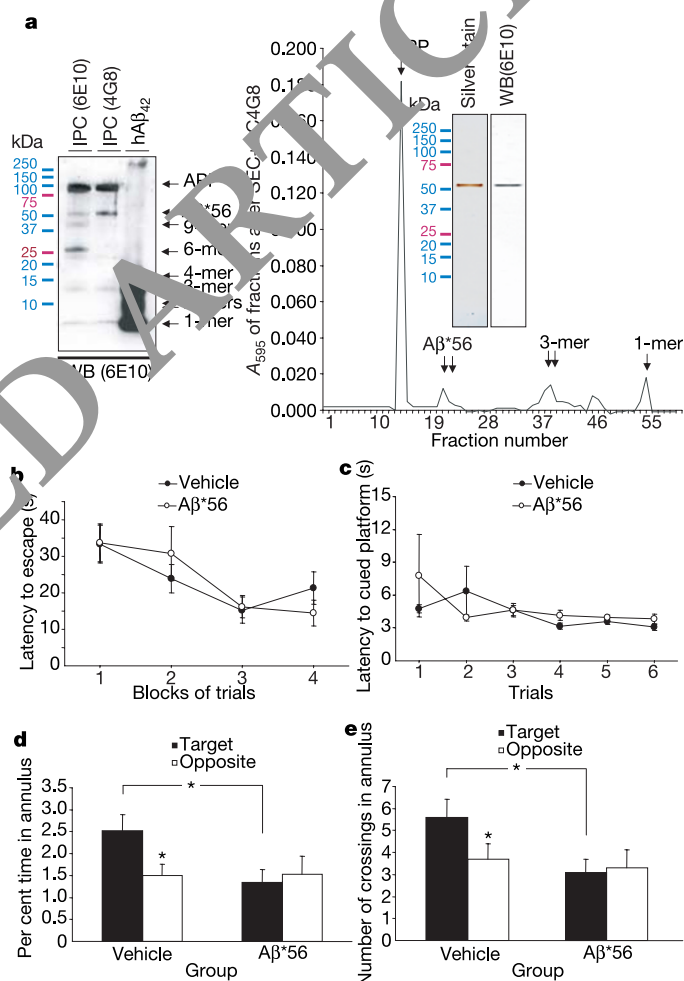
After a 24-h retention interval, rats received another injection of A $\beta^*$ 56 or vehicle, in the same manner as during training, and

underwent a probe trial two hours later during which the platform was absent. Spatial memory was shown by the vehicle-injected rats but not by those treated with A $\beta^*$ 56. There was a significant interaction of group  $\times$  quadrant ( $F_{1,18} = 6.288$ ,  $P = 0.022$ , analysis of variance (ANOVA)), but no main effects of group or quadrant (Fig. 4d). Rats in the vehicle group spent significantly more time in a target annulus surrounding the platform than in a control annulus in the opposite quadrant ( $t_9 = 2.668$ ,  $t$ -test with 9 degrees of freedom;  $P = 0.026$ ). In contrast, rats in the A $\beta^*$ 56 group spent similar amounts of time in both locations ( $P = 0.547$ ,  $t$ -test). In addition, rats in the A $\beta^*$ 56 group spent significantly less time in the target annulus than those in the vehicle group ( $t_{18} = 2.5$ ,  $P = 0.021$ ,  $t$ -test).

Other measures, including the number of crossings (Fig. 4e) and



**Figure 3 | Levels of the 56-kDa A $\beta$  assembly show the strongest inverse correlation with spatial memory.** **a–f**, Lack of significant correlations between retention of spatial memory and monomeric (4.5 kDa), trimeric (14 kDa) or hexameric (27 kDa) soluble A $\beta$  species detected in extracellular-enriched fractions of 5-month-old (open circles) and 6-month-old (filled circles) Tg2576 $^{+/-}$  mice. **g, h**, Levels of A $\beta$  assemblies corresponding to nonameric (40 kDa) and dodecameric (56 kDa) species correlate inversely with memory at 6 months of age (ANOVA).



**Figure 4 | Purification of A $\beta^*$ 56 from Tg2576 brain and effects of purified A $\beta^*$ 56 on memory in young rats.** **a**, Purification of total (RIPA-buffer-soluble) A $\beta$  species using immunoaffinity purification columns (IPC) packed with 200  $\mu$ g of 6E10 or 4G8 antibodies (left panel). Right panel shows absorbance at 595 nm ( $A_{595}$ ) of proteins isolated by affinity chromatography with 4G8 (AC4G8) that were separated by size-exclusion chromatography (SEC) to yield fractions containing A $\beta^*$ 56. **b**, There is no difference between rats that received A $\beta^*$ 56 and vehicle (50 mM ammonium acetate, pH 8.5) in terms of latency to locate a hidden platform during training. Data show blocks of two training trials each (mean  $\pm$  s.e.m.). **c**, There is no difference between rats (A $\beta^*$ 56 and vehicle treatment groups) in terms of latency to locate a visible platform. **d, e**, A $\beta^*$ 56 impairs spatial memory. Rats that received vehicle, but not A $\beta^*$ 56, injections showed a significant spatial bias for the escape location 24 h after training. Target annulus measures are shown in **d**, and number of annulus crossings in **e** (mean  $\pm$  s.e.m.). Two-way ANOVA ( $P < 0.05$ ) followed by  $t$ -test (\*,  $P < 0.05$ ),  $n = 10$  animals per group.



quadrant time, also provided no evidence of spatial bias as an indication of retention in the A $\beta$ \*56 group. Thus, A $\beta$ \*56 impaired long-term memory, but not acquisition of spatial information (Supplementary Discussion). Ten days later, both groups had equally good acquisition and retention in the same two-day protocol when new spatial information was once again provided but no A $\beta$ \*56 was administered, indicating no lasting debilitation as a result of treatment (Supplementary Fig. 6b, c). Notably, the behavioural effects of administering purified A $\beta$ \*56 to rats parallel exactly our previous observations in 6- to 11-month-old Tg2576 mice<sup>6</sup>, which show intact performance during the acquisition phase of the water maze training but impairment in probe trials after an overnight retention interval. Furthermore, in the current test, A $\beta$ \*56 had no effect on escape latencies to a visible platform (Fig. 4c), consistent with the lack of an A $\beta$ \*56 effect on performance of Tg2576 mice in the cued phase of the water maze training (Supplementary Fig. 3). Our data demonstrate that A $\beta$ \*56 impairs memory in healthy, young rats, and strongly support the hypothesis that A $\beta$ \*56 is the principal cause of memory decline in middle-aged Tg2576 mice.

The transient effects of A $\beta$ \*56 application in rats imply that A $\beta$ \*56 impairs memory by inducing transient physiological, rather than permanent neuropathological, alterations of the brain. The accumulation of insoluble aggregating proteins such as tau, huntingtin, prion protein, ataxin and A $\beta$  occurs in many neurodegenerative disorders. These aggregates often define the disorders neuropathologically, but their relative contribution to disease symptoms compared to other, hypothetical, intermediate protein assemblies is controversial<sup>21–23</sup>, and the identity of the theoretical intermediates has been elusive. Our finding that A $\beta$ \*56 is responsible for memory loss in plaque-forming Tg2576 mice, and causes memory deficits when injected directly into healthy rats, sets a precedent for identifying other 'star' proteins inducing brain dysfunction—for example, tau\* (ref. 22). That A $\beta$ \* is a highly specific form of A $\beta$  offers the potential for developing precise diagnostic methods to detect its correlate in humans with pre-clinical Alzheimer's disease, presenting the possibility of targeting A $\beta$ \* and aborting the disease before permanent structural changes have developed.

## METHODS

**Transgenic animals.** Tg2576 mice<sup>3</sup> were the offspring of mice backcrossed successively to B6SJL/F1 breeders.

**Behavioural tests.** Spatial reference memory was assessed in Tg2576 mice using a modified version of the Morris water maze<sup>6</sup>. Testing was tailored for Tg2576 transgene-positive and -negative mice in the B6SJL background strain. Tg2576 transgene-positive and -negative mice received visible platform training for three days (eight trials per day), followed by hidden platform training for nine days, with four trials per day (trials 30 s each; inter-trial interval (ITI) 20–40 min). Three probe trials (60 s) were performed 20 h after 12, 24 and 36 training trials (after days 3, 6, and 9 of training), and the mean per cent target quadrant occupancy for the three probe trials was calculated.

Long-Evans male rats, approximately 4 months of age (young adults), were used to test the effects of A $\beta$ \*56 infusions on memory. For pre-training, rats received three trials (90 s; 15 s each) per day for eight days, during which they were allowed to locate a camouflaged platform that remained in the same location 2 cm below the water surface in a 1.83-m diameter maze. Every sixth trial was a probe trial (30 s), which served to assess the development of a spatially localized search for the escape platform. Performances during the probe trials were used to generate a learning index as previously described<sup>24</sup>. Immediately after pre-training, unilateral cannulae were implanted into a lateral ventricle. Coordinates for the cannulae tip were 1.0 mm posterior to bregma, 1.5 mm lateral to midline, and 3.5 mm ventral to the skull surface. Rats were allowed two weeks to recover before being trained to a new platform location in a new water maze environment (with a different physical location and surrounding spatial cues). Ten rats received A $\beta$ \*56 and another ten received vehicle (50 mM ammonium acetate buffer, pH 8.5). Rats in the A $\beta$ \*56 and vehicle groups were matched for body weight and learning index in pre-training. Mean body weights on the training day for rats in A $\beta$ \*56 and vehicle groups were 509.6  $\pm$  24.43 g (mean  $\pm$  s.e.m.) and 525.1  $\pm$  19.10 g, respectively ( $t < 1$ ). Mean learning indices for rats in the A $\beta$ \*56 and vehicle groups were 202.3  $\pm$  10.9 and 214.8  $\pm$  12.4, respectively ( $t < 1$ ). A $\beta$ \*56 was injected at a concentration of 0.85  $\mu$ M. The injection volume

was 10  $\mu$ l (1.0  $\mu$ l min<sup>-1</sup>, with one additional minute at the end to allow diffusion away from the cannulae). An equal volume of buffer was injected into rats in the vehicle group. Two hours after injection, rats were trained in the water maze. Rats received eight training trials (90 s each; ITI 8 min) to locate a hidden escape platform. The entire training session was completed within 60 min. The retention interval was 24 h. Two hours before the probe trial, A $\beta$ \*56 or vehicle was infused as before training. The percentage of time spent in an annulus that was 1.5 $\times$  the size of the target platform was used to analyse performance in the probe trial. At the end of the probe trial (120 s), rats received six cued training trials to locate a visible escape platform. The location of this platform varied from trial to trial. Ten days after the test with A $\beta$ \*56, both groups were re-tested in the absence of any intraventricular injections before the training session (eight trials) or retention probe trial. The escape platform was in a new location and curtains with new spatial cues surrounded the maze. Both groups improved performance on training and had a similarly accurate spatial search for the correct platform location during the retention test 24 h later (Supplementary Fig. 6b).

**Antibodies.** The following primary antibodies were used: 6E10 (monoclonal raised against A $\beta$ <sub>1–17</sub>) and 4G8 (monoclonal raised against A $\beta$ <sub>17–24</sub>) (1:100–10,000 dilution; Signet Laboratories), R1732 (polyclonal raised against A $\beta$ ; 1:75 dilution)<sup>25</sup>, R1736 (polyclonal raised against residues 595–611 of APP695; 1:1,000 dilution)<sup>26</sup>, FCA3542 (polyclonal raised against A $\beta$ 42)<sup>27</sup>, APPCter-C17 (polyclonal raised against the carboxy terminus of APP; 1:5,000 dilution)<sup>28</sup>, anti-Flotillin-2, anti-ERKs, anti-JNK and anti-c-Jun (all at 1:200 dilution; Santa Cruz), anti-tau-5 (1:1,000 dilution; Biosource International), anti-MAP-2 (1:200 dilution; Sigma and Santa Cruz) and anti-actin (1:250 dilution; Sigma), and anti-tPA (anti-tissue plasminogen activator; American Diagnostica). The A11 anti-oligomer antibody (1:5,000 dilution)<sup>18</sup> was detected with a biotinylated anti-A11 antibody (1:2,000,000 dilution; Vector Laboratories) and ExtrAvidin (1:5,000 dilution; Sigma).

Protein extractions, protein concentration determination, immunoaffinity chromatography, size-exclusion chromatography, silver staining, liquid chromatography–tandem mass spectroscopy, western blotting and immunoprecipitation procedures are described in Supplementary Methods.

Received 23 November; accepted 13 December 2005.

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

**Acknowledgements** We thank S. Younkin, D. Walsh, M. Podlisny, D. Selkoe, J. Cleary and S. Prusiner for critical discussions. We are grateful to D. Cooper-Blacketer, J. McQuail and M. Sherman for technical help, and A. Delacourte and N. Sergeant for providing the APPC17-Cter antiserum. This work was supported by grants from the NIH (to K.H.A., M.G. and A.Y.) and a gift from M. and H. Hobbs to M.G.

**Author Contributions** S.L. and K.H.A. conceived the project. S.L. planned, performed and analysed the biochemistry experiments, including the protein extractions and purification of A $\beta$ \*56. K.H.A. wrote most of the paper. M.T.K. and M.G. planned, performed and analysed the rat behavioural experiments, and L.K. planned and analysed the mouse behavioural experiments. A.Y. provided the mass spectrometry analysis of purified A $\beta$ \*56. C.G.G. provided biochemistry advice, and C.G.G. and R.K. donated the A11 antiserum.

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