Immunization reverses memory deficits without reducing brain Aβ burden in Alzheimer's disease model

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We have previously shown that chronic treatment with the monoclonal antibody m266, which is specific for amyloid β-peptide (Aβ), increases plasma concentrations of Aβ and reduces Aβ burden in the PDAPP transgenic mouse model of Alzheimer's disease (AD). We now report that administration of m266 to PDAPP mice can rapidly reverse memory deficits in both an object recognition task and a holeboard learning and memory task, but without altering brain Aβ burden. We also found that an Aβ/antibody complex was present in both the plasma and the cerebrospinal fluid of m266-treated mice. Our data indicate that passive immunization with this anti-Aβ monoclonal antibody can very rapidly reverse memory impairment in certain learning and memory tasks in the PDAPP mouse model of AD, owing perhaps to enhanced peripheral clearance and (or) sequestration of a soluble brain Aβ species.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive loss of cognitive abilities and by neuropathological features including amyloid deposits, neurofibrillary tangles, and neuronal loss in selective brain regions. Amyloid deposits are extracellular proteinaceous aggregates characteristically seen in the associative cortices and limbic system, their principal constituent being a 39–43 amino acid peptide(s), the amyloid β-peptide (Aβ). Aβ peptides are derived from the processing of a larger transmembrane protein, the β-amyloid precursor protein (APP). It has been postulated that abnormal processing of APP to Aβ, or reduced clearance of Aβ from brain, resulting in the parenchymal aggregation of Aβ to form amyloid deposits is an early and crucial event in the pathogenesis of the disease[1–3]. One possible therapeutic approach is to target the enzymes that process APP to Aβ peptides, with the aim of reducing their synthesis or deposition. Other approaches are aimed at increasing the clearance of Aβ peptides from brain or inhibiting their aggregation into insoluble deposits. Among these latter approaches, both active and passive immunization against Aβ have recently been reported to prevent Aβ and amyloid deposition in transgenic mouse models of AD[4–10].

Active immunization can prevent or reverse both amyloid deposition and the associated memory impairment in transgenic mouse models of AD[7,8]. Passive immunization, consisting of prolonged treatment with monoclonal or polyclonal anti-Aβ antibodies, can prevent the development of amyloid deposits in PDAPP mice[5,9]. Because we have previously found age-dependent object recognition memory impairment in PDAPP mice[11,12], we first investigated whether or not subchronic passive immunization, using the monoclonal anti-Aβ antibody m266, could reverse object recognition memory deficits. In addition, we investigated whether acute administration of m266 could reverse memory impairment of PDAPP mice in the object recognition task as well as in a holeboard learning task. PDAPP mice administered m266 acutely or subchronically showed markedly improved learning and memory performance without any alteration in cortical or hippocampal Aβ burden (percentage of surface area covered by Aβ deposits), suggesting that a reduction in Aβ or amyloid deposition is probably not responsible for the improvements. We readily detected an Aβ/m266 native complex in the plasma and cerebrospinal fluid (CSF) of PDAPP mice after administration of m266, raising the possibility that enhanced peripheral clearance and (or) sequestration of Aβ in brain might underlie the rapid reversal of memory impairment that followed the antibody treatment.

Results

We have previously found age-dependent object recognition memory deficits in PDAPP mice when measuring long-term memory performance across several hours[11,13]. Accordingly, we initially selected this task[11] as a means to test whether or not relatively brief passive immunization with an anti-Aβ antibody

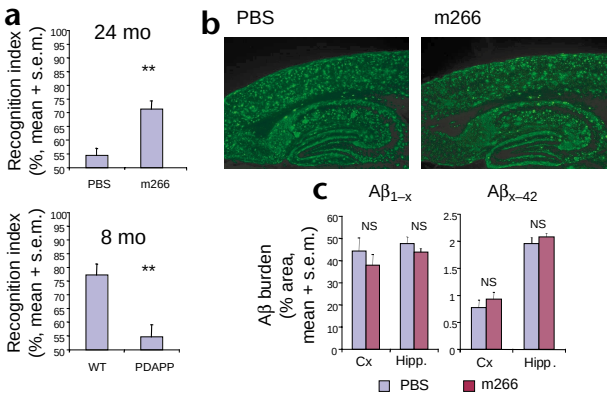

Fig. 1. Passive immunization reversed object recognition memory deficits, but did not reduce Aβ burden, in PDAPP mice. PDAPP transgenic male mice 24 months of age (n = 8 per group) were injected (i.p., 200 μl) with anti-Aβ antibody m266 (360 μg) or PBS once per week for 6 weeks. Behavioral testing was done 3 d after the last injection. (**a**) Performance in the object recognition task is expressed as a recognition index corresponding to the percentage of time spent exploring a novel object versus a familiar object during the test session (see Methods). A recognition index of 50% indicates that the two objects were explored similarly (the mice do not discriminate between the novel and the familiar object). Object recognition memory performance of 24-month PDAPP mice treated with m266 is significantly better (** $P < 0.01$, ANOVA) than that of mice treated with PBS (upper panel), and is very similar to object recognition memory performance of 8-month wild-type (WT) mice studied in a parallel experiment (lower panel, n = 8 per group). Note that 8-month PDAPP mice do not perform well in the object recognition task (lower panel). (**b**) 3D6-immunostained sagittal brain sections from 24-month PDAPP mice treated with PBS (left) or the m266 antibody (right). (**c**) Cortical as well as hippocampal Aβ burden does not differ between treatment groups. $Aβ_{1–x}$, Aβ peptides starting at residue 1 and 3D6-immunostained; $Aβ_{x–42}$, Aβ peptides ending at residue 42 and 21F12-immunostained; NS, not significant (ANOVA).

could reverse the behavioral deficits in PDAPP mice. In our first experiment, very old PDAPP mice (24 months of age) were treated for only six weeks with the anti-Aβ monoclonal antibody m266 (360 μg, intraperitoneally (i.p.), once per week) and then tested in the object recognition task. We found a highly significant group effect ($P < 0.01$) with regard to the object recognition index (**Fig. 1a**). In contrast to PBS-treated PDAPP mice, which explored novel and familiar objects to the same degree, m266-treated PDAPP mice spent more time exploring a novel object than a familiar object explored 3 hours earlier ($P < 0.01$). Notably, the performance of these24-month m266-treated PDAPP mice was nearly identical to that of younger (8-month) wild-type control mice (**Fig. 1a**). Treatment with the antibody had no effect on the total duration of object exploration, extent of locomotor activity or number of rearings (data not shown). Additionally, treatment with a purified mouse IgG_1 isotype control had no effect on the performance of PDAPP mice in the object recognition task (data not shown).

Recently, we reported that prolonged passive immunization (for five months) of PDAPP mice (four months of age at the beginning of treatment) with this same anti-Aβ antibody markedly reduces cortical Aβ burden[9], as has been recently reported for other Aβ monoclonal antibodies[5]. Notably, however, the areas occupied by Aβ deposits (the Aβ burden) in both the hippocampus and cerebral cortex of 24-month PDAPP mice after only six weeks of passive immunization did not differ from those in age-matched PBS-treated PDAPP con-

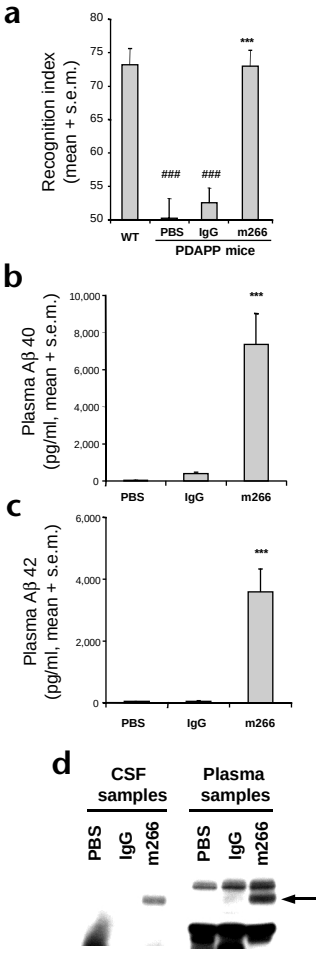

Fig. 2. Object recognition memory performance improved after acute administration of m266 antibody. PDAPP male mice 11 months of age were injected with m266 (360 μg, i.p.), a control IgG or PBS 3 h before the familiarization session, which corresponded to 24 h before trial 1. An additional group of untreated age-matched wild-type (WT) mice was tested in parallel. (**a**) PDAPP mice treated with either PBS (n = 7) or control IgG (n = 8) performed at chance levels (recognition index, 50%), whereas m266-treated PDAPP mice (n = 8) and age-matched wild-type mice (n = 6) performed above chance (within-group t-test analysis). Values are means + s.e.m. ***, $P < 0.0001$ versus PBS- and IgG–treated PDAPP groups; ###, $P < 0.0001$ versus wild-type mice. (**b**, **c**) After administration of m266, plasma concentrations of both $Aβ_{1–40}$ and $Aβ_{1–42}$ were significantly increased when compared to PBS- and IgG-treated mice ($P < 0.0001$), as detected by ELISA[22]. When compared to values for PBS-treated mice, the increase in plasma $Aβ_{1–40}$ in m266-treated mice was approximately 150-fold greater and the increase in $Aβ_{1–42}$ was approximately 70-fold greater. Neither plasma $Aβ_{1–40}$ nor $Aβ_{1–42}$ concentrations differed between PBS- and control IgG–treated mice. Values are means + s.e.m. ***, $P < 0.0001$ versus PBS and control IgG PDAPP groups. (**d**) Detection of a high-molecular-weight Aβ/m266 complex in cerebrospinal fluid and plasma 24 h after a single administration of m266 (360 μg). Samples were pooled for each treatment group, size fractionated under non-denaturing conditions and immunoblotted using biotinylated 3D6 antibody. In each lane, 4 μl of cerebrospinal fluid or 1.5 μl of plasma was loaded. An Aβ/antibody complex was readily detected in both the cerebrospinal fluid and plasma from m266-treated mice only. Arrow, Aβ/m266 complex.

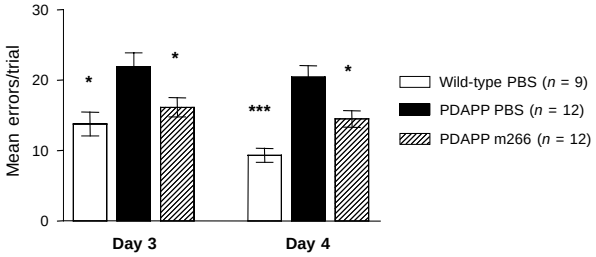

Fig. 3. Deficits in a holeboard learning task improved after acute administration of m266 antibody. Female PDAPP mice 11 months of age were used to test the effect of m266 in the holeboard learning and memory paradigm (see Methods). Mice were administered 360 μg (i.p.) of m266 or PBS 24 h before the first day of testing. Values are the mean number of errors per trial (± s.e.m.) for the last 2 days of testing. PDAPP mice (n = 12) made more errors on days 3 and 4 than did age-matched wild-type mice (n = 9). After a single dose of m266, PDAPP mice (n = 12) made fewer errors than did PBS-treated PDAPP mice. *, $P < 0.05$; ***, $P < 0.001$.

trols (**Fig. 1b**, **c**). Correlation analyses did not show any association between amyloid pathology and behavior ($r = -0.111$ and $r = 0.243$ for total $A\beta_{1-x}$ burden and $A\beta_{x-42}$ burden, respectively; NS). In addition, the Aβ burden measured in the hippocampus and cortex of very old m266-treated PDAPP mice was substantially greater than that in younger (8-month) untreated PDAPP mice (data not shown), who were completely unable to discriminate novel and familiar objects in the object recognition task (**Fig. 1a**).

These unexpected results led us to further investigate whether acute treatment with m266 could reverse object recognition memory deficits in relatively old PDAPP mice. We next studied the effect of m266 administered acutely (360 μg, i.p.) to PDAPP mice (11 months of age) 1 day before testing in the object recognition task. Here again, the object recognition index differed significantly between treatment groups (group effect: $F_{3,25} = 25.085$, $P < 0.0001$) (**Fig. 2a**). The m266-treated PDAPP mice performed significantly better on the object recognition task than did PBS- and control IgG–treated PDAPP mice ($P < 0.0001$), and performed comparably to age-matched wild-type mice. The object recognition index did not differ between PBS- and control IgG–treated PDAPP mice. In addition, a within-group t-test analysis confirmed that m266-treated PDAPP mice (t-value = 9.526, $P < 0.0001$) and wild-type mice (t-value = 9.581, $P < 0.0002$) performed above chance values (50%), whereas PBS- and control IgG–treated PDAPP mice did not (t-values = 0.081 and 1.157 respectively, NS). A group-effect analysis also confirmed that there were no differences between groups in total object exploration time during trial 1 ($F_{3,25} = 0.555$, NS) or trial 2 ($F_{3,25} = 0.679$, NS). The distance traveled also did not differ between groups during the familiarization session (group × block interaction, $F_{15,125} = 1.455$, NS; group effect, $F_{3,25} = 1.200$, NS), during trial 1 (group effect, $F_{3,25} = 1.326$, NS) or during trial 2 (group effect, $F_{3,23} = 1.334$, NS).

We next investigated whether passive immunization with m266 could also reverse learning and memory deficits of PDAPP mice measured in a holeboard task. We have recently found that PDAPP mice have cognitive deficits in this task as compared to wild-type control mice (unpublished data). In the present study, 11-month PDAPP mice made more errors than did age-matched wild-type controls across 4 days of testing; (group effect, $F_{1,19} = 23.067$, $P < 0.0001$). However, learning performance improved markedly in PDAPP mice treated with a single dose of m266 (360 μg, i.p.) as compared to those treated with PBS. The m266-treated mice made significantly fewer errors than did the PBS-treated mice on days 3 and 4 of testing (**Fig. 3**; group effect, $F_{1,22} = 9.053$, $P = 0.006$).

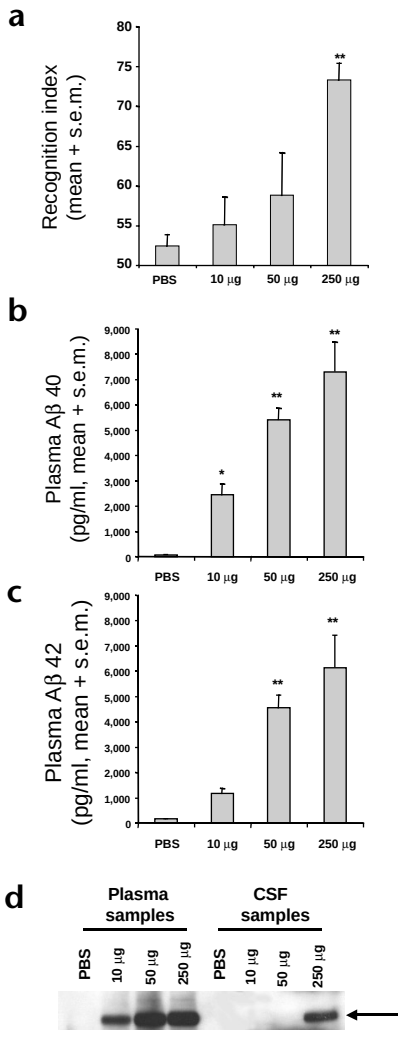

Fig. 4. Dose-dependent effect of m266 on object recognition performance, Aβ plasma levels and m266/Aβ complex formation. (**a**) Male PDAPP mice 12 months of age were used to establish a dose–response relationship for m266 treatment (PBS, 10 μg, 50 μg and 250 μg, i.p.) in the object recognition task. PDAPP mice treated with PBS (n = 8), 10 μg (n = 8) or 50 μg (n = 8) of m266 performed at chance levels, whereas PDAPP mice treated with 250 μg of m266 (n = 8) performed above chance (within-group t-test analysis). Values are means + s.e.m. **, $P < 0.01$ versus the other groups. (**b**, **c**) Rapid, marked increases in plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations occur in a dose-dependent fashion after m266 treatment. Plasma Aβ concentrations do not differ between groups treated with 50 or 250 μg of m266. Values are means + s.e.m. *, $P < 0.05$ versus PBS; **, $P < 0.01$ versus PBS. (**d**) Detection of a high-molecular-weight Aβ/m266 complex in cerebrospinal fluid and plasma 24 h after administration of a single dose of m266. Samples were pooled for each group, size fractionated under non-denaturing conditions and immunoblotted with biotinylated 3D6 antibody. For each lane, 8 μl of cerebrospinal fluid or 4 μl of plasma were loaded (see Methods). Arrow, Aβ/m266 complex.

We next investigated the dose–response relationship for m266 treatment on object recognition memory performance, and determined the presence or absence in plasma and CSF of an Aβ–antibody complex. Administration of various doses of m266 to PDAPP mice resulted in a significant difference between treatment groups on the object recognition index (group effect: $F_{3,28} = 7.39$, $P < 0.001$) (**Fig. 4a**). The performance of PDAPP mice administered 250 μg of m266 was significantly better than the performance of PDAPP mice administered either PBS or 10 or 50 μg of m266 ($P < 0.01$). The object recognition index did not differ between PBS-treated PDAPP mice and PDAPP mice administered 10 or 50 μg of m266. In addition, a within-group *t*-test analysis confirmed that PDAPP mice administered 250 μg of m266 (*t*-value = 10.935, $P < 0.0001$) performed above chance levels (50%), whereas the other groups did not.

We saw a dose-dependent increase in the plasma concentrations of both $A\beta_{1–40}$ and $A\beta_{1–42}$ peptides after acute m266 treatment (**Fig. 4b**, **c**). A significant increase in the concentration of plasma $A\beta_{1–40}$ occurred for all doses ($P < 0.0001$), with no significant difference between the two highest doses (50 and 250 μg). A significant increase in plasma $A\beta_{1–42}$ occurred when PDAPP mice were administered 50 or 250 μg of m266, and the effects of these two doses did not differ significantly from each other. When proteins from plasma and CSF samples were size-fractionated by gel electrophoresis under native conditions (**Fig. 4d**), an antibody/Aβ complex could readily be detected in all plasma samples from PDAPP mice treated with m266. In contrast, a complex could only be detected in CSF samples from PDAPP mice treated with the highest dose of m266 (250 μg), which was the lowest dose found to be effective in reversing learning and memory impairment in this experiment. Notably, concentrations of both the $A\beta_{1–40}$ and $A\beta_{1–42}$ peptides in brain extracts (hippocampus or cortex) did not differ between groups at any dose of m266 (data not shown).

Discussion

We have shown that subchronic (six weeks) passive immunization with an antibody directed towards the central domain of Aβ, which was previously shown to reduce amyloid burden in PDAPP mice after chronic administration[9], reverses memory impairment in an object recognition task in 24-month PDAPP mice. We also found that the near-normal performance in the object recognition task was not associated with reduced Aβ deposition in either the cerebral cortex or hippocampus. Consequently, we tested whether acute (single-administration) passive immunization with m266 would also reverse cognitive impairment in PDAPP mice, and found rapid reversal of memory impairment in both the object recognition task and a hole-board learning task. Improved learning and memory performance was not associated with any detectable alteration in brain Aβ burden.

Our data seem to contrast with previous studies, which have suggested that the reduction in cognitive impairment seen in APP transgenic mice after active immunization requires reduced brain Aβ/amyloid burden. Indeed, active immunization can prevent the development of AD-like neuropathology[4,6] as well as preventing memory impairment in several mouse models of AD[7,8]. In one study, active immunization with Aβ of double mutant APP TgCRND8 mice (K670N/M671L and V717F) partially prevented the development of reference memory deficits in a water maze task[7]. But there was only a 50% reduction in the size and number of dense core amyloid deposits, and active immunization had no effect on the total insoluble pool of Aβ in brain. The researchers concluded, however, that the prevention of memory deficits was due to the reduced amyloid pathology seen in their immunized mice. In a second study, chronic active immunization had similar beneficial effects on memory impairment in two different strains of transgenic mice as assessed using a radial-arm water maze[8]. Notably, treatment did not affect amyloid pathology in the same way in the two strains of mice. Immunized Tg2576 APP+PS1 double transgenic mice showed a reduction in diffuse (nonfibrillar) Aβ deposits in the cerebral cortex and hippocampus, but not in amyloid (fibrillar Aβ) deposits. In contrast, Tg2576 APP transgenic mice showed a small but statistically significant reduction in cortical amyloid burden, suggesting that active immunization reduces the development of fibrillar Aβ deposits in this mouse strain. These authors concluded that active immunization prevents memory deficits by altering either brain amyloid pathology or an unknown pool of non-deposited Aβ, perhaps a soluble pool of Aβ. Taken together with our data, these results suggest that the relationship between soluble and insoluble brain Aβ concentrations and memory impairment in transgenic mice is task specific and complex.

We have previously reported that chronic passive immunization with the m266 antibody reduces brain Aβ burden in PDAPP mice at least in part by altering brain Aβ clearance[9]. In m266-immunized mice, plasma Aβ concentrations increase markedly[9] and can be completely depleted when samples are treated with protein G, indicating that the antibody binds to and completely sequesters plasma Aβ. In addition, we have previously shown that m266 does not decorate amyloid deposits in PDAPP mice after peripheral administration[9]. These data suggest that m266 can reduce Aβ burden by facilitating Aβ efflux or clearance from brain. In the present study, we also saw pronounced increases in plasma Aβ concentrations after m266 treatment, and could readily detect an Aβ/m266 complex in both plasma and CSF. It should be noted that an Aβ/m266 complex was readily detected in CSF only at the two highest doses of m266 (250 μg and 360 μg) shown to be effective in the object recognition memory task. Thus, it is also conceivable that at these doses, m266 may enter the central compartment and sequester soluble Aβ. Several previous studies have shown that direct intracerebral administration of Aβ disrupts memory in rodents (for review, see ref. 14). Alternatively, treatment with m266 may enhance the clearance of Aβ from brain to plasma[9], thereby reducing a central pool of Aβ responsible for memory impairment.

Regardless of the exact mechanism, it is clear that the reversal of memory impairment by m266 in these two memory tasks occurs too quickly to be due to an effect on brain Aβ burden. In addition, in extensive studies, no clear correlation between deposited forms of Aβ (such as plaques) and memory impairment in AD patients has been demonstrated. In fact, recent reports suggest that soluble Aβ may be better correlated to both neurodegeneration and memory impairment[15–17]. Further studies will be required, however, to fully determine the mechanism by which m266 affects memory performance. Our data show that m266 may bind and sequester soluble Aβ in blood and brain, but not plaque-deposited Aβ[9], and rapidly reverse memory impairment in two behavioral paradigms in PDAPP mice. If soluble Aβ is involved in the memory impairment associated with AD, as suggested by several recent studies[15–17], then passive immunization with certain antibodies may rapidly reduce cognitive impairment in AD patients apart from any effect it may have on amyloid deposition.

METHODS

Mice. All experiments were done in compliance with protocols approved by the Eli Lilly and Company Institutional Animal Care and Use Committee. PDAPP mice are derived from a hybrid background representing combinations of C57BL/6J, DBA/2J and Swiss-Webster strains[18]. Mice were handled daily for 5 d before behavioral testing began. All mice had free access to food and water, except on the nights before testing in the holeboard task, during which they were deprived of food. Mice were housed at a room temperature of 23 ± 1°C and with a light/dark cycle of 12 h:12 h with lights-on at 6:00 a.m. Behavioral experiments were carried out during the light period, between 8:00 a.m. and 2 p.m. Different sets of mice were used for each experiment. The monoclonal anti-Aβ antibody m266 (ref. 19) and a purified mouse IgG_1 isotype control (Pharmingen, San Diego, California) were diluted from stock solutions in PBS before administration.

Object recognition task. This task, based on the spontaneous tendency of rodents to explore a novel object more often than a familiar one[20,21], was performed as described previously[11]. On the first day of testing, mice were submitted to a 30-min familiarization session in the empty open field. On the next day, mice were submitted to two 10-min trials with a 3-h inter-trial delay. During trial 1, mice were allowed to explore the open field in the presence of object A (marble or die). During trial 2, mice were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object B (die or marble). A recognition index, calculated for each mouse, was expressed as the ratio $(T_B \times 100)/(T_A + T_B)$, where T_A and T_B are the time spent during trail 2 on object A and object B, respectively. The distance traveled (cm) was recorded using a computer-assisted video tracking system (San Diego Instruments, San Diego, California), and the time spent exploring the object (nose pointing toward the object at a distance ≤1 cm) was recorded by hand by an observer blind to the treatment status of the mice.

Holeboard memory task. This task measured a mouse's ability to remember which 4 out of 16 equidistant holes were baited with food. It was carried out in test chambers (44.5 × 44.5 × 30.5 cm; Med Associates, St. Albans, Vermont) containing a holeboard floor insert. During 4 consecutive days of testing, mice were given four 180-sec trials per day with a 2-min inter-trial interval. For all trials, the same holes were baited with access to a single 20-mg food pellet (no. F0021, BioServe, Laurel, Maryland). To control for olfactory cues, all of the holes were baited with food pellets placed beneath a screen that prevented access to the pellets. Mice were deprived of food each night before testing. Testing was always done during the first half of the day, and free access to food was provided 1 h after the last mouse was tested. Two arrays of infrared beams were used to track activity on the floor and nose-pokes into the holes. Extra-maze cues were provided in the form of various items of hardware, computer cables and a single house light, all mounted in a standardized fashion inside sound-attenuated cubicles containing the test chambers. Before each mouse was tested, test chambers were cleaned with distilled water, dried thoroughly and had clean floors inserted. A global measure of cognitive performance was determined by calculating the average number of errors per trial that a mouse made each day. An error consisted of entering a hole that was never baited (reference memory error), re-entering a baited hole (working memory error) or not entering a baited hole (error of omission).

Immunohistochemistry. Immunohistochemical analyses were carried out on 24-month PDAPP mice after completion of the object recognition task. Mice were anesthetized with avertin (0.023 ml per gram body weight) and perfused transcardially with heparinized saline. Brains were drop-fixed in 4% paraformaldehyde and embedded in paraffin. Serial sagittal sections 8 μm thick were cut using a Leica (Bannockburn, Illinois) RM 2135 microtome and placed on poly-L-lysine-coated slides (Fisher Scientific, Pittsburgh, Pennsylvania) with two sections per slide. Every fifteenth pair of sections was immunoreacted with one of the following monoclonal antibodies: 3D6 (specific for the free amino-terminal region of Aβ; 1:500) or 21F12 (specific for $Aβ_{x–42}$; 1:1,000). The specificity of the immunoreactivity was confirmed by pre-adsorption with the appropriate peptides as well as by checking that no signal was detected when the primary antibody was omitted. Analysis of immunoreactive deposits for Aβ was done on a PC using the public-domain program NIH Image J (http://rsb.info.nih.gov/nih-image), by defining a region of interest and setting a threshold to discriminate non-specific staining. The percentage of surface area covered by Aβ immunoreactivity was used to measure Aβ burden. Two regions were analyzed: an area of parietal cortex comprising layers I–VI and an area of the hippocampal formation comprising layers oriens, pyramidal layer, stratum radiatum and the dentate gyrus.

Immunoassays and immunoblotting. Biochemical analyses were carried out on the groups of mice tested in the object recognition paradigm after a single administration of m266. After the completion of trial 2, mice were anesthetized with avertin. CSF samples were collected from the cisterna magna using a 50-μl Hamilton syringe with a 33-gauge needle. Blood samples were collected by cardiac puncture into EDTA tubes. Plasma was separated by centrifugation at 6,000 rpm (Tomy MTX-150 centrifuge, with TMA-11 rotor, TOMY Tech, Palo Alto, California) for 10 min. After cardiac puncture, mice were transcardially perfused with cold heparinized saline for 2 min. Brains were rapidly removed from the skull, and cortices and hippocampi were dissected and immediately frozen on dry ice. Brains were processed using a three-step extraction procedure; each step was followed by centrifugation at 10,000 rpm (same centrifuge) for 10 min at 4°C. In the first step, samples were homogenized in cold PBS with proteinase inhibitors (Complete, Boehringer-Mannheim, Indianapolis, Indiana). In the second step, the pellet was resuspended in RIPA buffer (50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP40, 0.1% SDS and Complete, pH 8.0). In the third step, the pellet was resuspended in 5 M guanidine-HCl and the sample tubes were rocked for 2 h at room temperature. $Aβ_{1–40}$ and $Aβ_{1–42}$ in each pool were quantified using an enzyme-linked immunosorbent assay (ELISA)[22]. Briefly, the monoclonal antibodies 2G3 and 21F12 (10 μg/ml each) were used to capture Aβ peptides terminating at residues x–40 and x–42, respectively[23]. Biotinylated 3D6 (0.5 μg/ml), which recognizes the $Aβ_{1–5}$ region of human Aβ, was used as the reporter antibody.

Proteins from plasma and CSF samples were separated by electrophoresis under non-denaturing conditions using a 4–20% polyacrylamide/TBE gel (Criterion gel, Bio-Rad, Richmond, California) and transferred to PVDF membranes, with 10 mM CAPS, 0.01% SDS and 1% methanol, pH 11, used as transfer buffer. After pre-incubation in SuperBlock blocking buffer (Pierce, Rockford, Illinois), the membrane was probed with biotinylated 3D6 (0.05 μg/ml), reacted with streptavidin (1:200,000) and visualized using SuperSignal West Femto (Pierce).

Statistical analyses. To compare object recognition data as well as the plasma and CSF Aβ concentrations between groups, one-way or two-way analyses of variance (ANOVA) were done using StatView software, version 5 (SAS Institute, Cary, North Carolina). Holeboard learning data were analyzed by repeated-measures ANOVA (using Systat version 10; SPSS, Chicago) and two-tailed *t*-tests for *post hoc* comparisons.

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Competing interests statement

The authors declare competing financial interests; see the Nature Neuroscience website (http://neuroscience.nature.com) for details.

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