Correlation between cognitive deficits and Aβ deposits in transgenic APP+PS1 mice


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Abstract

Doubly transgenic mAPP1-mPS1 mice (15–16 months) had impaired cognitive function in a spatial learning and memory task that combined features of a water maze and a radial arm maze. Nontransgenic mice learned a new platform location each day during 4 consecutive acquisition trials, and exhibited memory for this location in a retention trial administered 30 min later. In contrast, transgenic mice were, on average, unable to improve their performance in finding the hidden platform over trials. The cognitive performance of individual mice within the transgenic group were inversely related to the amount of Aβ deposited in the frontal cortex and hippocampus. These findings imply that mAPP1-mPS1 transgenic mice develop deficits in cognitive ability as Aβ deposits increase. These data argue that radial arm water maze testing of doubly transgenic mice may be a useful behavioral endpoint in evaluating the functional consequences of potential AD therapies, especially those designed to reduce Aβ load. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Alzheimer’s disease (AD) is characterized by progressive, severe cognitive deficits which include impaired memory for recent events at an early stage of the disease process. Two pathologic characteristics of the disease are Aβ peptide containing, congophilic amyloid plaques and neurofibrillary tangles [49]. Although the extent to which either of these causes the dementia of Alzheimer’s disease remains unresolved, both have been correlated with the degree of cognitive decline in dementia [11,14,15,18,28,38,46]. One of the first to demonstrate a relationship between amyloid and cognitive function was Blessed et al. [3]. However, this was disputed as primarily arising from an overall group difference between control and demented patients; the correlation disappeared when control cases were excluded [50]. In this study, synapse loss was argued to be the best corre-
cases [27]. Mice expressing mutant forms of the amyloid precursor protein (mAPP) develop Aβ deposits that resemble those found in AD [24,33]. When mice expressing mAPP are crossed with mice expressing a mutant form of presenilin 1 (mPS1), the rate of deposition is accelerated by a factor of 2 to 4 [5,30,31,43]. Thus far, mice expressing mPS1 transgenes alone have not been found to deposit Aβ, although brain concentrations of the long Aβ variant(s) are elevated [6,10,21].

Behaviorally, mAPP+mPS1 transgenic mice have been observed to have intact acquisition and retention in the Morris water maze when tested at 6 or 9 months of age, in spite of increasing Aβ load in cortex and hippocampus [1,31]. Age-related increases in brain Aβ deposits have been reported for several transgenic models of AD [24,25,33,36,49]. In some transgenic lines behavioral effects of the transgenes have been reported [9,32,33,39]. Some of these behavioral deficits appear ontogenically before the histologic deposition of Aβ has started [12,20,32,37,39]. Thus far, a correlation between declines in behavioral performance and the amount of Aβ deposition within individual transgenic mice (excluding controls) has not been reported. The present study examined whether cognitive impairments could be reliably detected in mAPP + mPS1 transgenic mice, and whether the amount of Aβ pathology in each transgenic mouse was correlated with the extent of its behavioral impairment.

2. Materials and methods

2.1. Animals

Ten doubly transgenic PS1<sub>M146L</sub> + APP<sub>K670N,M671L</sub> (3 males and 7 females; PS1 line 5.1, ref 21; APP line Tg2576, ref 33) and 11 nontransgenic litter mates (4 males and 7 females) were tested when 14.5 to 16 months of age; mean 15.5). The Tg2576 mice derive from a C57B6/SJL X C57B6 background, while the PS1–5.1 line derives from a Swiss Webster/B6D2F1× B6D2F1 background, producing progeny with a mixture of these backgrounds. After weaning, animals were genotyped by slot blot analysis of tail DNA and group housed until several weeks prior to behavioral testing, at which time they were individually housed. All animals had free access to water and rodent chow and were maintained on a 12-h light/dark cycle. All behavioral testing was done in the light phase. Prior to water maze testing, a sensorimotor test battery was administered to verify mice were not blind, deaf or neurologically impaired [31,39].

2.2. Radial arm water maze testing

The maze consisted of a 100 cm circular pool with 6 swim arms radiating from a common circular swim area (similar to that described for rats; ref [17]). On each of 9 training days, a submerged escape platform was positioned near the end of a goal arm (goal arm location was moved each day). On each day, there were 4 consecutive acquisition trials of 1 min each (designated T1 through T4). For each acquisition trial, the mouse was placed in a start arm for that trial (which was never the goal arm). The start arm sequence for each day’s acquisition trials was chosen randomly except that no start arm was used for more than one acquisition trial. The number of errors (arm entries that did not result in finding the platform) was recorded. If the animal made an error, it was pulled back into the start arm for that trial to permit another choice to be made. For each testing day, “error reduction” between T1 and T4 was used as an within-day index of learning. Following T4, the animal was returned to its home cage for 30 min, and then given a single retention trial (designated T5), with the start arm being the same as T4 for that day. On a small number of trials (<2%), mice did not make any arm entries. When this occurred, these values were excluded from the statistical analyses. All mice used in this study were physically capable of swimming through the maze.

2.3. Histopathology

Within 1 week of completing radial arm water maze testing mice were anesthetized with pentobarbital (100 mg/kg i.p.), perfused transcardially with 25 ml normal (0.9%) saline, the brains were rapidly removed, and the left hemispheres were immersion fixed in freshly depolymerized 4% paraformaldehyde (buffered to pH 7.4 in potassium phosphate) for 24 h, then cryoprotected in a series of sucrose solutions. Fixed, cryoprotected hemispheres were frozen and sectioned in the horizontal plane at 25 μm using a sliding microtome and stored at 4°C in Dulbecco’s phosphate-buffered saline (PBS) for immunocytochemistry and histology. Immunocytochemistry was performed on floating sections as described in detail previously [26]. The “total Aβ” primary antiserum was raised against Aβ1–40 in rabbits and recognizes both Aβ1–42 and Aβ1–40 in ELISA assays, and was blocked by preabsorption with either peptide, or Aβ1–16. Antisera selective for Aβ ending at residue 40 and Aβ ending at residue 42 were purchased from Quality Controlled Biochemical (Hopkinton, MA). Selectivity was confirmed by preabsorption experiments, which blocked all punctate staining. Sections were incubated with the primary antibody overnight at 4°C, then incubated in the biotinylated secondary antibody (2 h) followed by streptavidin-peroxidase. Peroxidase reactions consisted of 1.4 mM diaminobenzidine with 0.03% hydrogen peroxide in PBS for exactly 5 min. Nonspecific reaction product formation was negligible as assessed by omitting the primary antibody and/or preincubating antisera with Aβ. The low background staining seen in nontransgenic mice confirmed the near absence of nonspecific antibody reactivity. Each assay was balanced with respect to the experimental groups.

Congo red histology was carried out using sections
mounted on slides and air dried for a minimum of 12 h, then rehydrated for approximately 30 s before staining. Hydrated sections were incubated in an alkaline alcoholic saturated sodium chloride solution (2.5 mM NaOH in 80% reagent alcohol [95% ethanol, 5% isopropanol], freshly prepared) for 20 min, then incubated in 0.2% Congo red in alkaline alcoholic saturated sodium chloride solution (freshly prepared and filtered) for 30 min. Sections were rinsed through three rapid changes of 100% ethanol, cleared through three changes of xylene, then coverslipped with Permount.

2.4. Image analysis

Immunocytochemistry or Congo red staining of tissue sections was quantified with an Oncor V150 image analysis system. The software uses hue, saturation and intensity (HSI) to segment objects in the image field. Operationally, thresholds for object segmentation are established using a series of standard slides which have the extremes of intensity for the stain being measured. Thresholds in HSI space are established which accurately identify objects on all standard slides and these segmentation thresholds remain constant throughout the analysis session. Section to section variability in immunostaining is minor owing to rigid fixation and staining protocols and the HSI segmentation parameters accurately identify positive staining on all sections. The operator was unaware of the behavioral score or genotype when measurements were taken. Staining in the frontal cortex and the hippocampus were quantified from 4 horizontal sections from each mouse spaced 600 μm apart beginning at 2000 μm ventral to bregma. The starting sections from each mouse were matched as closely as possible. The frontal cortex measurement used a 80 × field with one limit of the field of view resting on the edge of the cortex and the other limit resting the midline in the most anterior position possible. The measurement area was a rectangular video field of 850,000 μm² (0.85 mm² located within the center of that field of view. The measurement area was primarily of the middle 2/3rd of the cortical mantle (i.e. not including laminae 1 or 6). Hippocampus used the standard boundaries of the fimbria anteriorly, ventricle medially and a large number of smaller, less intensely stained deposits. The Aβ40 antisera label both the congophilic deposits and a substantial number of Aβ deposits restricted to hippocampus and cerebral cortex in young mice [30,31], these deposits were also found in striatum, thalamus and brain stem of 15 months old transgenic mice. In cerebral cortex, there was a general correspondence between birefringent Congo red stained amyloid deposits (Fig. 2D) and Aβ deposits stained in serial sections with antisera for Aβ peptides ending at residue 40 (Aβ40; Fig. 2B), residue 42 (Aβ42; Fig. 2C) and total Aβ (Fig. 2A). The total Aβ and Aβ42 antisera label both the congophilic deposits and a large number of smaller, less intensely stained deposits. The Aβ40 antisera stain the congophilic deposits heavily, but are less capable of revealing the small, less intensely stained deposits (Fig. 2).

When measured by image analysis, the doubly transgenic mice had total Aβ loads in frontal cortex that averaged 52%, with a range from 28 to 64%. In the hippocampus, the total Aβ load in the transgenic mice averaged 25% (range 10 to 42%). The values for Congo red staining were 20 fold less than the total Aβ load (range 2.0 to 3.3% in frontal cortex). The values for Aβ42 load were similar to those for total Aβ, while the Aβ40 load ranged 5–10%.

3.2. Radial arm water maze performance

The radial arm water maze has walls within the water tank which direct the mice to swim into one of six swim paths (arms) in a manner similar to arm entries in the dry radial arm maze (Fig. 3). Each day, mice had the opportunity to learn which arm contained the hidden platform on 4 consecutive swim trials (T1 through T4). The hidden platform was always located in the same arm on each trial within a day and in a different arm across days. The difference between T1 and T4 errors provided a simple means with which to quantify within-day learning for each animal. Within-day retention was tested on T5, which took place 30 min after T4 was completed.

Fig. 4 summarizes the performance of both nontrans-
genic and transgenic mice on this task from the time they were naive (Block 1) until they were well-trained (Block 3). In the first block, during which all mice were first learning the procedural components of the task, (i.e. that there was an escape platform located at the end of an arm and that the platform location was constant within a day), there were no

Fig. 1. Regional distribution of Aβ deposition in 16 month old mice. No Aβ deposition is found in the nontransgenic mice (panel A). In APP+PS1 mice (panel B), there is a high density of deposits in hippocampus, cortex and striatum, with smaller numbers of deposits appearing in thalamic and brainstem structures. The cerebellum lacks parenchymal Aβ deposits, although vascular deposits are visible at high magnification. Sections from one hemisphere were collected in the horizontal plane. cbl = cerebellum, ecx = entorhinal cortex, hc = hippocampus, pcx = pyriform cortex, str = striatum, thal = thalamus. Scale bar equals 2 mm.

Fig. 2. Colocalization of Aβ immunohistochemistry with Congo red staining in frontal cortex. Four adjacent sections were stained with antisera to total Aβ (panel A) Aβ40 (panel B), Aβ42 (panel C) or Congo red (panel D, viewed with cross-polarization). Four discrete deposits (arrowheads) are found in the same relative location in each panel, suggesting they are the same deposits. Scale bar equals 200 μm.
effects of genotype or trials on the number of errors. In block 2, there was a significant effect of trials (F(2, 28) = 6.5, P < 0.01), but not of genotype, indicating that, in general, the animals were improving their performance from T1 to T4 and T5. By the end of training (Block 3), the nontransgenic mice had clearly acquired the task, with most mice making 0 or 1 errors on T5. ANOVA indicated a significant effect of trials (F(2,28) = 3.5; P < 0.05), genotype (F(1,29) = 9.1; P < 0.005) and a trials by genotype interaction (F(2,26) = 3.5; P < 0.05). Tukey-Kramer comparisons indicated that there was a significant difference between the transgenic and nontransgenic mice on T5 (P < 0.01; asterisk on Fig. 4). Thus, by the third block of training days the control mice were able to learn and remember the within-day location of the hidden platform. The transgenic mice, by contrast, showed no evidence of improvement over trials on either T4 or T5 in the third block of trials.

3.3. Correlations between histopathology and radial arm water maze performance

Given that there were cognitive deficits found in the transgenic mice, we evaluated whether the extent of Aβ deposition was related to the degree of cognitive impairments in individual mice. In transgenic mice, the Congo red staining in frontal cortex was positively correlated with the number of errors they committed on T5 (r = 0.74, P < 0.05; Fig. 5A). A similar correlation was observed with cortical Aβ40 load and errors on T5 (r = 0.83; P < 0.01; Fig. 5B). Hence, the extent of cortical pathology correlated with the magnitude of the retention trial impairment.

Analysis of the learning index (T1 errors–T4 errors) with the histopathology revealed a relationship between total Aβ load in the frontal cortex and hippocampus with learning. There was a significant negative correlation between acquisition and total Aβ load in frontal cortex (r = −0.74; P < 0.05; Fig. 6A) and hippocampus (r = −0.70; P < 0.05; Fig. 6B). Therefore, greater total Aβ load in these two brain areas correlated with a greater impairment of cognitive function in the transgenic mice.

4. Discussion

The clear differences in behavioral performance between two groups compared here indicate the radial arm water maze task is valuable in distinguishing the cognitive performance capacities of transgenic and nontransgenic mice. The feasibility of imposing the radial maze alleyways onto a swim tank was first described by Buresova et al. [7], using a win-shift strategy. Recently, Diamond et al. [17], demonstrated that the win-stay approach (used in the present study) was a very sensitive index of rodent working memory impairment produced by stress. This variant of the water maze paradigm has several advantages over the more widely used reference memory (fixed platform location) version of this task [31,39]. First, the relocation of the platform forces mice to solve the task using working memory. In the traditional water maze task [45], the constant platform location each day simplifies the task and permits the slow acquisition of static information. Second, the ability to use errors rather than escape latency diminishes the impact of swimming performance (ability/speed) on the values used to assess learning. This may prove particularly useful in evaluating older animals, where variations in swim speed may be considerable, independent of memory skills. Third, performing the retention trial on the same day as the repeated acquisition trials permits evaluation of memory functions on a time scale (less than an hour) similar to the registration and recall tests used clinically to diagnose the early stages of Alzheimer’s disease. A final advantage of this task is that it can be performed longitudinally, permitting identification of the age at which each mouse begins to exhibit acquisition and/or retention deficits. The cognitive
deficits found here in the transgenic mice suggest this task will be useful in screening therapeutic approaches expected to reduce cognitive dysfunction in AD.

Our earlier work with mAPP+mPS1 mice identified a deficiency in Y maze alternation that appeared prior to the first Aβ deposits [30,31]. Interestingly, at 15–16 months, doubly transgenic mice no longer exhibit deficits in Y maze alternation [1], suggesting this task is unlikely to be useful in screening therapeutic approaches intended for AD. By contrast, doubly transgenic mice are not impaired in radial arm water maze performance at 5–7 or 11.5 months of age [1,44], but are impaired in the working memory aspect of radial arm water maze performance by 15.5 months. Thus, age-related cognitive impairment in this task occurs considerably after the appearance of Aβ pathology in the same transgenic model, and only after a significant accumulation of Aβ has occurred, providing the opportunity to determine the relationship between behavior impairment and Aβ pathology.

The main outcome of this study was a strong correlation between the extent of Aβ neuropathology in aging transgenic mice and their behavioral impairment in the radial arm water maze. Specifically, fibrillar Aβ deposit area correlated with the number of errors on the retention trial in individual transgenic mice. Whether measured by Congo red staining or by Aβ40 immunostaining, the area occupied by compacted, fibrillar amyloid was strongly associated with this index of memory performance. We emphasize that these correlations did not depend upon the inclusion of control (nontransgenic) mice. These results imply that some transgenic mice (those with the lowest Aβ levels) were demonstrating at least partial learning of the platform location, even though the average performance of the group did not improve over trials.

The distribution of Aβ40 and Aβ42 immunoreactivities reported here was very similar to that found in Alzheimer’s and Down’s syndrome brains [34,35,48]. Alzheimer’s disease researchers have a range of opinions regarding the pathogenicity of fibrillar versus nonfibrillar Aβ, including some who argue that Aβ accumulation is epiphenomenal. The results presented here are consistent with the view that fibrillar Aβ can interfere with cognitive function. However, they do not rule out the possibility that fibrillar deposits might, alternatively, act as an index of the rate at which Aβ is being produced in that animal, and that all pools of Aβ (soluble, dimeric, oligomeric, intracellular) covary with this readily detectable marker. Possible actions associated with Aβ that might cause such interference are degeneration of synapses [24,51], formation of dystrophic neurites [41,43], induction of inflammatory reactions [2,23], loss of LTP [9], or selective degeneration of memory-critical neurons [8].

The present correlations between memory and histopathology within the transgenic mice were made possible by the broad range of values for both Aβ pathology and be-
behavioral performance, permitting detection of correlations in a relatively small group of mice. The association found here is consistent with an earlier study in aged dogs, where a correlation was found between brain Aβ accumulation and acquisition of a visual discrimination task [29]. These results are similarly consistent with the finding that Aβ load correlates with intellectual status in humans, (although, in some studies, other measures of AD pathology; e.g. synapse density, tangle distribution; correlate better; [3,11,13–15,18,28,38,46,50]).

In addition, total Aβ load was correlated with the learning index in transgenic mice. Even though transgenic mice on average did not learn the task, some did improve more than others, as shown in Figs. 5 and 6. This correlation occurred in both the hippocampus and frontal cortex, while the correlation of fibrillar Aβ with trial 5 retention performance was only found in frontal cortex. Typically tasks designed to test working memory are thought to be hippocampus dependent, and one might have predicted a greater role for hippocampal Aβ. Importantly, the correlation for hippocampal fibrillar Aβ and trial 5 retention was positive, but not significant statistically. Additionally, water maze tasks appear to involve rapid consolidation, as memory for the hidden platform location becomes stable to electroconvulsive shock between 15 and 30 s post-trial [4]. Perhaps this rapid consolidation indicates a greater role of cortical structures in short-term retention of water maze tasks.

The total Aβ load measurement includes both fibrillar (compacted) and presumably nonfibrillar, diffuse Aβ deposits. Given that the amount of Congo red staining is 1/20th of the staining area labeled with antibodies to Aβ (similar to that in human brain, ref [16]), we assume the bulk of the
total Aβ staining was in the non-Congophilic diffuse form. Thus the correlation between learning deficits and total Aβ load in the transgenic mice could represent interference of nonfibrillar Aβ with neural function. This is consistent with the data that intracranial (i.c.) injections of Aβ peptides can cause amnesia in mice and rats [22,40,42]. It also complements the recent data from Dodart et al. [19], finding that object recognition deficits in PDAPP mice occurred even on the APO E null background, which fail to deposit fibrillar Aβ.

In conclusion, these results indicate that deposition of Aβ and formation of congophilic deposits were associated with declining cognitive function in 16 month old mAPP + mPS1 transgenic mice. The significant relationship between behavioral measures and Aβ pathology in individual mice reinforces the advantages of using the radial arm version of the water maze. Together with our additional work [1,31,44], the data indicate that behavioral deficits develop over time as the transgenic mice accumulate increasing amounts of Aβ. These data support the use of the radial arm water maze to evaluate the functional consequences of potential treatments for AD in transgenic mouse models.

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