Neurobiology of Disease

Treatment with an Amyloid-β Antibody Ameliorates Plaque Load, Learning Deficits, and Hippocampal Long-Term Potentiation in a Mouse Model of Alzheimer's Disease

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PDAPP transgenic mice overexpress a mutant form of human amyloid precursor protein under control of the platelet-derived growth factor promoter in CNS neurons that causes early onset, familial Alzheimer's disease in humans. These mice, on a mixed genetic background, have been shown to have substantial learning impairments from early ages, as well as an age-dependent decline in learning ability that has been hypothesized to be caused by amyloid-β (Aβ) accumulation. The goals of this study were to determine: (1) whether PDAPP mice on a pure C57BL/6 background develop more severe age-dependent learning deficits than wild-type mice; (2) if so, whether Aβ accumulation accounts for the excessive decline in learning ability; and (3) whether the learning deficits are reversible, even after significant Aβ deposition. At 4–6, 10–12, or 17–19 months of age, PDAPP and littermate wild-type mice on a C57BL/6 background were tested on a 5 week water maze protocol in which the location of the escape platform changed weekly, requiring the mice to repeatedly learn new information. PDAPP mice exhibited impaired spatial learning as early as 4 months (pre-Aβ deposition), and the performance of both wild-type and PDAPP mice declined with age. However, PDAPP mice exhibited significantly greater deterioration with age. Direct evidence for the role of Aβ accumulation in the age-related worsening in PDAPP mice was provided by the observation that systemic treatment over several weeks with the anti-Aβ antibody 10D5 reduced plaque deposition, increased plasma Aβ, improved hippocampal long-term potentiation, and improved behavioral performance in aged PDAPP mice with substantial Aβ burden.

Key words: **APP; amyloid; spatial; immunization; electrophysiology; neuropathology**

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of aging. It is characterized by memory loss and a gradual decline in general cognitive abilities. Neuropathological hallmarks include extracellular amyloid plaques [composed predominantly of aggregated forms of the amyloid-β (Aβ) peptide], intracellular neurofibrillary tangles (NFTs), microglial activation, neuronal loss, and synaptic loss. In humans with AD, the cognitive and behavioral disturbances seen in AD do not appear to become evident until years after significant neuropathology, including a large amount of plaques, has accumulated, and cell death has occurred (Goldman et al., 2001; Morris and Price, 2001). PDAPP transgenic mice express human amyloid precursor protein (APP) with a mutation (V717F) that causes an autosomal dominant form of familial AD (FAD). Expression of the APP transgene is under control of the platelet-derived growth factor promoter, leading to the generation of human Aβ within the CNS. In both humans and mice, the mutation leads to increased production of $A\beta_{42}$, which is more susceptible to fibril formation than the more abundant $A\beta_{40}$. Similar to humans with AD, PDAPP mice show an age-related accumulation of diffuse and neuritic plaques beginning at ~6–9 months of age, glial activation, and abnormal phosphorylation of cytoskeletal proteins.

Unlike humans with FAD, PDAPP mice do not develop NFTs or overt neuronal loss, and they have learning deficits before plaque deposition (from a few months of age, as early an age as they have been tested) (Dodart et al., 1999; Chen et al., 2000). Because these early deficits occur well before significant amounts of Aβ have accumulated or have changed into a more toxic β-sheet conformation, it is possible that they are caused by overexpression of human APP or any of the derivatives of APP that may influence cell function. It has also been shown that these mice, when on a mixed genetic background, not only have spatial learning deficits when young, but also an age-dependent worsening in spatial learning (Chen et al., 2000). It has been suggested that the age-dependent learning deficits in these mice are attributable to the effects of Aβ aggregation, although this has not been proven. The goals of this study were to determine: (1) whether PDAPP mice on a pure C57BL/6 background develop more severe age-dependent learning deficits than wild-type (WT) mice;

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(2) if so, whether Aβ accumulation plays a role in the learning deficits; (3) whether the learning deficits are reversible, even after significant Aβ deposition; and (4) whether there are electrophysiological correlates of the learning deficits, and whether they are they reversible.

Materials and Methods

Animals

PDAPP transgenic mice in which the platelet-derived growth factor promoter is used to drive expression of human APP with a mutation that causes FAD (APP^{V717F}) (Games et al., 1995) and their WT littermates (all on a C57BL/6 genetic background) were housed three to five to a cage and maintained on *ad libitum* food and water with a 12 h light/dark cycle. Separate groups were tested in the water maze at 4–6 months (young; little to no plaque deposition), 10–12 months (middle-aged; with some plaque deposition), or 17–19 months (old; with high levels of plaque deposition) of age. Approximately equal numbers of male and female mice were used.

To help determine whether Aβ deposition was responsible for any cognitive deficits in the old PDAPP mice, another group of 17- to 19-month-old PDAPP mice was tested in the water maze after treatment with the anti-Aβ antibody 10D5, a mouse monoclonal antibody that binds to Aβ amino acids 3–6 (Hyman et al., 1992; Bacskai et al., 2002) and is specific for human Aβ. PDAPP mice were given weekly intraperitoneal injections of 0.5 mg of 10D5 or saline (volume, 0.2 ml), and WT littermates were given saline injections. To determine whether Aβ deposition was responsible for any cognitive deficits in the old WT mice, another group of 22- to 24-month-old WT mice was tested in the water maze after treatment with the anti-Aβ antibody β-amyloid monoclonal-91 (BAM91; Sigma, St. Louis, MO), a mouse monoclonal antibody directed against amino acids 13–28 of Aβ. It recognizes soluble murine and human Aβ, stains plaques, and does not recognize APP on Western blots.

Water maze

The water maze has been very useful for studying age-related changes in learning and memory in mice (Morris, 2001). This test of spatial navigation learning requires the mouse to find a hidden (submerged) platform in a pool of water using visual cues from around the room. An analysis of mouse behavior in the water maze (Janus, 2004) showed that as a mouse's ability to find the platform improves, its average swim time and path length generally decrease. The water maze consisted of a metal pool (diameter, 118 cm) in a well lit room filled to within 10 cm of the upper edge with water made opaque by the addition of white nontoxic tempera paint. The pool contained a round platform (diameter, 11 cm) that the mice could step on to escape the water. For each trial, a mouse was released nose against the wall into the pool at one of four release points and allowed to find the platform. All trials lasted a maximum of 60 s, at which point the mouse was manually guided to the platform. An overhead camera recorded the animals' swim paths, allowing for quantification of distance, latency, and swimming speed by a computer (Polytrack; San Diego Instruments, San Diego, CA).

Cued water maze. The cued test was used to assess sensorimotor and/or motivational deficits that could affect performance during the spatial water maze task. For this task, the surface of the escape platform was visible (5 mm above the surface of the water), and a 10-cm-tall pole capped by a red tennis ball was placed on top of the platform to make its location even more obvious. The walls of the room were kept bare, although the experimenter and computer system were obvious spatial cues. The mice were given four consecutive trials per day, each time with the platform in a different location. The mouse was released into the pool opposite the location of the platform for that trial. After each trial, the mouse was placed into a holding cage for 30 s while the platform was moved to its next location. Cued testing continued for 5 d, giving each mouse a total of 20 cued trials.

Across all age groups, ~5% of WT mice and 15% of PDAPP mice displayed behaviors inappropriate for water maze testing (including spinning, thigmotaxic navigation around the perimeter of the pool, and inability to swim). These mice were removed from further study, leaving sample sizes of: 19 young WT, 15 young PDAPP, 21 middle-aged WT, 13 middle-aged PDAPP, 19 old WT, and 11 old PDAPP. This resulted in a relatively homogenous group of mice that presumably could see, swim, and were motivated to escape the water for continued testing in the spatial condition.

For the 10D5-treated group, cued water maze testing was administered 3 d after the first injection. A total of three PDAPP plus saline and five PDAPP plus 10D5 mice were removed from the study because of poor cued performance, leaving sample sizes of: 20 WT, 13 PDAPP plus saline, and 10 PDAPP plus 10D5.

Spatial water maze. Three days after the conclusion of cued testing, spatial testing began. For this task, the surface of the escape platform was submerged 1 cm below the surface of the water, and a variety of spatial cues were added to the walls of the room, requiring the mice to find the platform based on its relationship to the cues rather than direct visualization. Four consecutive trials were administered per day for 5 d. For this phase of testing, the position of the platform remained constant for all 5 d. Each day, the mouse was released once from each of four release points. Three days after the last day of testing, the mice were given a "probe" trial in which the platform was removed from the water maze, and the mice were allowed to search the pool for 30 s. The amount of time spent searching the quadrant that had contained the platform was measured, as well as the total number of times that the mouse crossed over the former location of the platform. One hour later, the platform was placed back into the pool in a different location, and 5 more days of spatial testing were administered using the new location. A total of five spatial locations were tested over the course of 5 weeks.

The spatial task was modified in an attempt to make it easier for the 10D5/saline-treated groups. A larger escape platform was used (diameter of 22 vs 11 cm), many more spatial cues were hung on the walls to make a more salient visual environment, and the platform remained in the same position for the duration of the experiment. Four trials (two consecutive trials, a 2 h break, and two more consecutive trials) were administered per day for 5 d. Forty-eight hours later, the next dose of 10D5 was administered. The mice were given a probe trial 24 h later in which the platform was removed from the water maze, and the mice were allowed to search the pool for 60 s. The platform was then replaced in the same position, and the testing/injection regimen was repeated two more times. Thus, the 10D5/saline-treated mice were tested for a total of 15 d in the modified spatial condition in which the position of the platform remained constant except for the probe trials.

Spontaneous locomotor activity

The activity levels of young and old PDAP mice were monitored for 1 h as described by Hartman et al. (2001) [Hamilton-Kinder (San Diego, CA) motor monitor].

Histological and biochemical analysis

At the completion of water maze testing, the mice were anesthetized, and ~0.5 ml of blood was withdrawn through the retro-orbital socket using heparinized capillary tubes. The blood was spun at 14,000 rpm for 5 min to separate the plasma, which was then frozen at −80°C for later analysis of human $A\beta_{40}$ and $A\beta_{42}$ levels by ELISA. The mice were then perfused through the heart with PBS, and their brains were removed. The left hemisphere of each brain was immersed in 4% paraformaldehyde in 0.1 M PBS at 4°C for 24 h and then soaked in a 30% sucrose solution at 4°C for 24 h, followed by freezing in powdered dry ice. The brain was then cut coronally in 50 μm sections from the genu of the corpus callosum to the end of the hippocampal formation. A subset of the sections (every sixth) was stained with pan anti-Aβ antibody (Biosource International, Camarillo, CA), and another subset of every sixth section was stained for fibrillar Aβ using thioflavine-S, as described previously (DeMattos et al., 2002). The hippocampus of each animal was assessed for Aβ and fibrillar Aβ load (i.e., percentage of area covered by deposits) using an unbiased stereological method (area fraction fractionator; Stereo Investigator; MicroBrightField, Colchester, VT) and a Nikon (Tokyo, Japan) E800 microscope. The hippocampus was dissected from the right hemisphere of each brain and frozen at −80°C for later analysis of levels of both carbonate soluble and insoluble human $A\beta_{40}$ and $A\beta_{42}$ by ELISA as described previously (DeMattos et al., 2002).

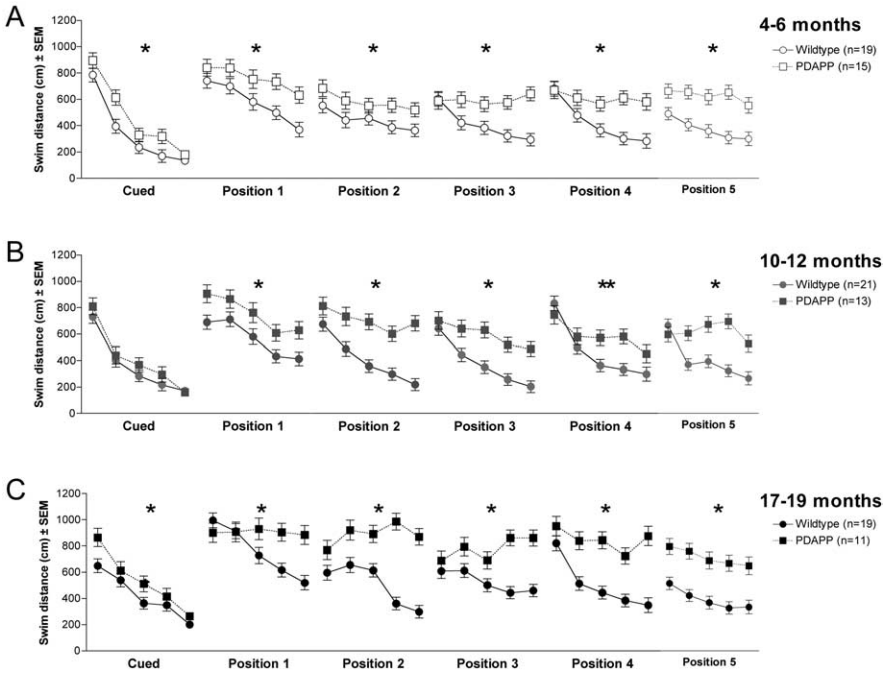

Figure 1. PDAPP mice on a C57BL/6 background exhibit profound spatial learning deficits from an early age. Performance was assessed by distance to find the visible platform (cued) versus the hidden platform (spatial positions 1–5) in the water maze. Each point represents the average of four daily trials. Significant main effects of genotype are indicated by *, and a significant genotype-by-day interaction is indicated by **. Error bars represent SEM.

The 10D5/saline-treated groups were similarly processed, except that a random subset of the 10D5/saline-treated group was killed for electrophysiological studies of long-term potentiation (LTP) in the hippocampus.

Electrophysiology

Mice were anesthetized deeply with halothane and decapitated, and the brain was removed. Hippocampi were rapidly dissected and placed in gassed (95% O_2-5% CO_2) standard extracellular solution containing the following (in mM): 124 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$ 1.25 NaH_2PO_4, 22 $NaHCO_3$, and 10 D-glucose. Transverse slices (500 μm thick) were cut with a vibratome (WPI, Sarasota, FL). Slices were then maintained in an incubation chamber for 1 h at 30°C in the standard solution. Individual slices were transferred to a submersion recording chamber, in which they were constantly perfused with standard solution (2 ml/min) at 30°C.

Extracellular recordings in gassed standard extracellular solution containing the following (in mM): 124 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$ 1.25 NaH_2PO_4, 22 $NaHCO_3$, and 10 D-glucose were obtained from the dendritic layer of the CA1 region with the use of 5–10 MΩ glass electrodes filled with 2 M NaCl. A bipolar electrode was placed in stratum radiatum to stimulate the Schaffer collateral/commissural pathway. Stimuli 50 μs in duration were applied every minute. The stimulus intensity was set to evoke 40–50% of the maximal amplitude of field EPSPs. Different types of afferent stimulation were performed at the same relative intensity in individual slices. To induce LTP, theta burst stimulation (six trains of six bursts of six pulses, each burst at 250 Hz, with 200 ms between bursts and 20 s between trains) (Errington et al., 1997; Chapman et al., 1999) was delivered. Field EPSPs were monitored and analyzed with the use of a computer-based data acquisition system. The magnitude of potentiation was expressed as the percentage of change in the maximal slope of EPSPs. Potentiation of the EPSP slope by >20% 60 min after theta burst stimulation was considered to represent successful induction and maintenance of LTP. Only a single slice from each hippocampus was used for each group of experiments.

Statistical analysis

Statistica 6.0 (StatSoft, Tulsa, OK) was used to analyze the collected data. An α-level of 0.05 was used for all statistical significance tests. All significant main effects and interaction effects were further tested using Tukey's honestly significant difference *post hoc* test for unequal *n*. No gender effects were found. Histological and biochemical data were analyzed using a one-way ANOVA with one between-subjects factor (age; young vs middle-aged vs old). To account for the pseudorandom nature of the distance to the escape platform from any given release point, swim path distance, escape latency, and swim speed data for the cued and each of the spatial platform locations were analyzed by averaging trials into blocks of four daily trials. These blocks were analyzed with two-way ANOVAs that included one between-subjects variable (group; WT vs PDAPP or WT vs PDAPP plus saline vs PDAPP plus 10D5) and one within-subjects variable (day; first through fifth). To avoid violating the assumptions of compound symmetry and sphericity that underlie univariate statistics for repeated-measures factors with more than two levels (i.e., differences between levels of repeated measures must not be correlated across subjects), the reported *p* values for every repeated-measures analysis reflect the Huynh-Feldt adjustment to the degrees of freedom. To assess whether acquisition performance improved significantly over each phase of training, one-way ANOVAs with one within-subjects factor (day; first vs fifth) were computed for each group separately. Probe trial data (time spent searching the probe quadrant and number of platform location crossings) for each phase were analyzed with one-way ANOVAs that included one between-subjects variable (group).

Results

Spatial learning in PDAPP mice: impairment that worsens with age

On the cued task (visible platform), WT and PDAPP mice of all ages exhibited significant improvements in performance across the test days (Fig. 1). Although young (4–6 months) and old (17–19 months), but not middle-aged (10–12 months), PDAPP mice learned the task more slowly than WT mice ($p < 0.0001$ and 0.02, respectively), all ages had reached WT levels of performance by the final day of cued testing. Additionally, there were no significant cued performance differences among the age groups of either genotype (Fig. 2). These results suggest that all of the mice in the experiment could see the platform, could swim, and were motivated to escape the water.

On the spatial learning task (place/submerged platform), WT mice performed well across all five platform positions, showing evidence of significant improvement at all ages ($p < 0.001$) (Fig. 1). On two of the five platform positions, old WT mice were slightly, but significantly, impaired compared with young and middle-aged WT mice ($p < 0.02$) (Fig. 2*A*). Compared with WT mice, PDAPP mice were significantly impaired at all ages across all platform positions (Fig. 1). For young and old PDAPP mice, the main effect of genotype was significant across all five platform positions ($p < 0.003$). For middle-aged PDAPP mice, the main effect of genotype was significant for all but one of the platform positions ($p < 0.004$), although a genotype-by-day interaction revealed significant impairment for that position as well ($p < 0.02$). The performance of young and middle-aged PDAPP mice did not differ, and both ages showed evidence of significant improvement on two of the five positions ($p < 0.05$) (Fig. 2*B*). Old

PDAPP mice, however, were impaired (compared with young and middle-aged PDAPP mice) on three of the five platform positions ($p < 0.05$) and did not exhibit evidence of significant improvement over the week of testing on any of the positions.

When the spatial learning swim distance data were averaged across all five platform positions (Fig. 3), PDAPP mice performed worse than WT mice across all age groups (main effect of genotype; $p < 0.0001$), and old mice performed worse (main effect of age; $p < 0.0002$) than young or middle-aged mice (which did not differ). Importantly, an interaction between genotype and age ($p < 0.05$) revealed that the performance of old PDAPP mice declined more with age than that of old WT mice. Interestingly, old mice of both genotypes swam significantly faster than young mice (data not shown). Therefore, if escape latency was the only measure assessed, one would conclude that PDAPP mice did not worsen with age to a greater extent than WT mice. This emphasizes the importance of assessing multiple variables when using the water maze. In the probe trials (data not shown), PDAPP mice of all three age groups failed to show any spatial bias for any of the platform positions, whereas WT mice consistently spent approximately one-third to one-half of the probe trial searching the quadrant that had previously contained the escape platform. It is possible that a shorter interval between the last acquisition trial and the probe trial would have facilitated recall in the PDAPP mice. Another alternative is that the learning demonstrated by PDAPP mice may not represent acquisition of spatial memory per se but may represent the ability to learn a strategy to better accomplish the task, as discussed by Janus (2004).

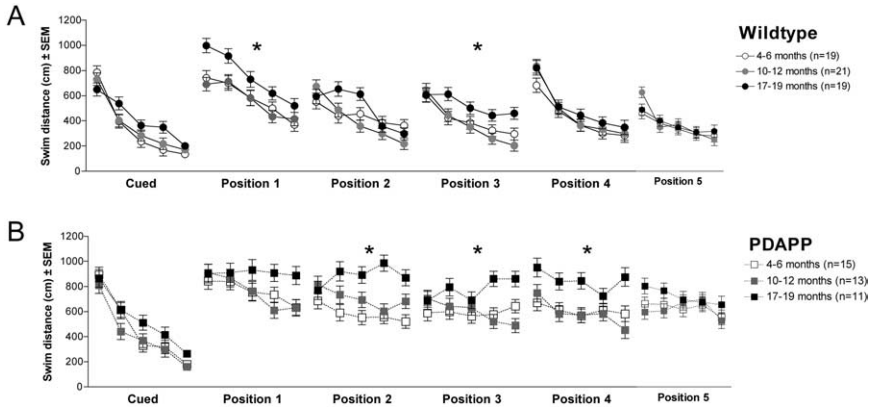

Figure 2. Old mice of each genotype perform worse than young or middle-aged mice in the spatial version of the water maze. Each point represents the average of four daily trials. Significant main effects of age are indicated by *. Error bars represent SEM.

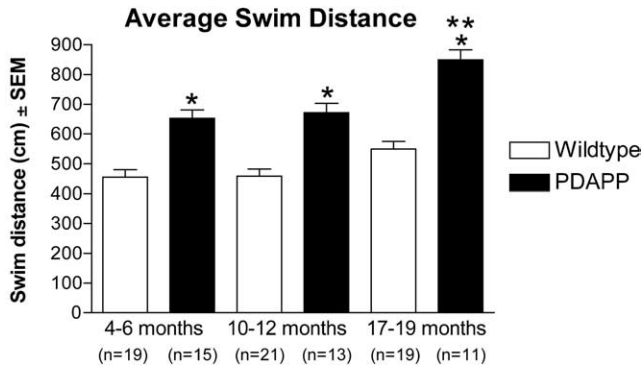

Figure 3. PDAPP mice developed more severe age-dependent spatial learning deficits than wild-type mice. Average swim distance across all five platform positions was assessed. Each bar represents the average performance across all platform positions. Significant main effects of genotype are indicated by *, and a significant genotype-by-age interaction is indicated by **. Error bars represent SEM.

To summarize the water maze results, both WT and PDAPP C57BL/6 mice performed quite well on the cued (visible platform) phase of the test. WT mice learned the spatial task (place/submerged platform) very easily, whereas PDAPP showed only minimal evidence of learning the task. Both WT and PDAPP mice showed evidence of decreased performance with age, but the performance of PDAPP mice declined much more severely, suggesting that age-related Aβ buildup may have negative consequences for spatial learning. The spontaneous locomotor activity levels of both WT and PDAPP mice decreased with age ($p < 0.0001$). Levels of activity were not significantly different between genotypes at either young or old ages, but there was a significant age-by-genotype interaction, in that PDAPP mice did not show as much of a decline in activity as WT mice ($p < 0.03$). There was no correlation between 1 h spontaneous activity and water maze performance.

Anti-Aβ antibody decreases plaque load and improves learning

As predicted from previous studies (Games et al., 1995; Johnson-Wood et al., 1997), analysis of Aβ load in the hippocampus (Fig. 4*A*) revealed significantly more staining ($p < 0.0002$) in old PDAPP mice than in young or middle-aged PDAPP mice (which did not differ significantly). Analysis of thioflavine-S staining in the hippocampus (Fig. 4*B*) also revealed significantly more thioflavine-S+ (fibrillar Aβ) deposits in middle-aged PDAPP mice than in young PDAPP mice ($p < 0.0004$) and more deposition in old PDAPP mice than in middle-aged PDAPP mice ($p < 0.0005$). ELISA measurements of hippocampal carbonate-soluble and -insoluble $A\beta_{40}$ and $A\beta_{42}$ revealed significantly more of each in old PDAPP mice (Table 1), but there were no differences in measurements of $A\beta_{40}$ and $A\beta_{42}$ in plasma (data not

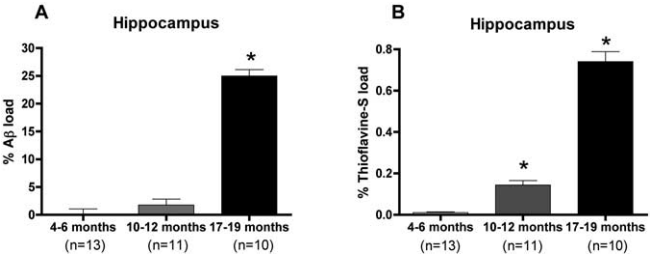

Figure 4. Both Aβ (***A***) and thioflavine-S+ Aβ (***B***) load increased with age in PDAPP mice. Significant main effects of age are indicated by *. Error bars represent SEM.

Table 1. ELISA hippocampal Aβ levels (pg/μg protein; means ± SE)

Assay	Young	Old	*p*
PDAPP carbonate-soluble $A\beta_{40}$	0.29 ± 0.03	2.95 ± 0.67	0.0002
PDAPP carbonate-insoluble $A\beta_{40}$	1.21 ± 0.15	67.23 ± 15.56	0.00008
PDAPP carbonate-soluble $A\beta_{42}$	0.38 ± 0.05	14.97 ± 3.17	0.00003
PDAPP carbonate-insoluble $A\beta_{42}$	4.81 ± 0.65	667.89 ± 143.61	0.00003
WT carbonate-soluble $A\beta_{40}$	0.07 ± 0.01	0.09 ± 0.01	NS
WT carbonate-insoluble $A\beta_{40}$	0.11 ± 0.02	0.13 ± 0.02	NS
WT carbonate-soluble $A\beta_{42}$	0.03 ± 0.003	0.04 ± 0.004	NS
WT carbonate-insoluble $A\beta_{42}$	0.12 ± 0.01	0.16 ± 0.01	NS

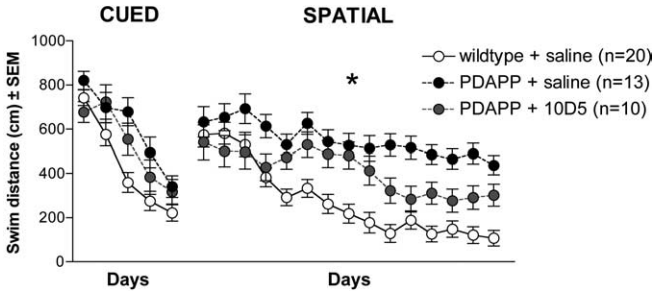

Figure 5. Treatment with 10D5 improved the spatial learning performance of aged PDAPP mice. Each point represents the average of four daily trials. Significant main effects of age are indicated by *. Error bars represent SEM.

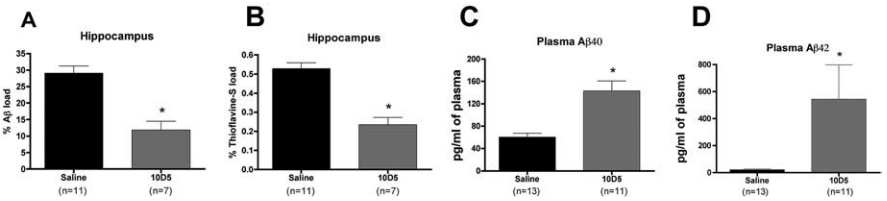

Figure 6. Treatment with 10D5 reduced Aβ (***A***) and thioflavine-S+ Aβ (***B***) load and increased $A\beta_{40}$ (***C***) and $A\beta_{42}$ (***D***) levels in the plasma of aged PDAPP mice. Significant main effects of age are indicated by *. Error bars represent SEM.

Table 2. ELISA Aβ levels (means ± SE)

Assay	PDAPP plus saline	PDAPP plus 10D5	*p*
Plasma $A\beta_{40}$[a]	60.5 ± 7	143.2 ± 17.6	0.0001
Plasma $A\beta_{42}$[a]	21.9 ± 5.5	544 ± 253.9	0.04
Carbonate-soluble hippocampal $A\beta_{40}$[b]	10.27 ± 7.22	8.88 ± 1.71	NS
Carbonate-insoluble hippocampal $A\beta_{40}$[b]	276.59 ± 122.23	190.13 ± 41.15	NS
Carbonate-soluble hippocampal $A\beta_{42}$[b]	37.82 ± 8.35	34.98 ± 5.38	NS
Carbonate-insoluble hippocampal $A\beta_{42}$[b]	1,275.15 ± 743.33	939.25 ± 232.44	NS

[a]Picograms of protein per milliliter.

[b]Picograms of protein per microliter.

shown). We found no age-dependent change in endogenous murine Aβ when comparing young versus old C57BL/6 WT mice (Table 1). Yao et al. (2004) did find a gender- and age-dependent increase in endogenous murine Aβ in mice when comparing mice expressing apoE3, apoE4, and apoE knock-out mice but did not compare wild-type C57BL/6 mice in their study.

Because of the observation that old PDAPP mice exhibit marked impairments in spatial navigation coincident with high levels of Aβ and thioflavine-S staining, we wanted to determine whether treatment with a reagent that specifically targets Aβ, the anti-Aβ antibody 10D5, would reverse either of these effects in old PDAPP mice. 10D5 is specific for human Aβ and does not recognize murine Aβ. On the cued (visible platform) task, WT plus saline mice performed slightly better ($p < 0.003$) (Fig. 5) than both PDAPP plus saline and PDAPP plus 10D5 mice (which did not differ). However, by the fifth day of testing, both PDAPP plus saline and PDAPP plus 10D5 mice had reached WT plus saline levels of performance.

Analysis of learning curves in the spatial learning task revealed that all groups exhibited significant improvement ($p < 0.001$) (Fig. 5). WT plus saline mice performed significantly better than both PDAPP plus saline and PDAPP plus 10D5 mice ($p < 0.03$). Interestingly and importantly, PDAPP plus 10D5 mice performed significantly better than PDAPP plus saline mice ($p < 0.02$). This suggests that Aβ is contributing to the age-dependent worsening of learning in PDAPP mice. On the probe trials, WT plus saline mice performed significantly better than PDAPP plus saline or PDAPP plus 10D5, which did not differ, and all groups showed an increased spatial bias to the probe quadrant over the three probe trials (data not shown). In a separate experiment, we also treated old WT mice with the anti-Aβ antibody BAM91 that recognizes murine Aβ and assessed animals in the cued and spatial task of the water maze. There were no differences in the performance of WT mice treated with either BAM91 or saline.

In regard to the biochemical and histological effects of 10D5, PDAPP plus 10D5 mice had significantly less Aβ and thioflavine-S staining ($p < 0.0002$) (Fig. 6*A*,*B*) and higher levels of both human $A\beta_{40}$ and $A\beta_{42}$ in the plasma ($p < 0.04$) (Fig. 6*C*,*D*) than PDAPP plus saline mice. Although levels of hippocampal carbonate soluble or insoluble human $A\beta_{40}$ or $A\beta_{42}$ were lower in PDAPP plus 10D5 mice, the effect was not significant (Table 2).

Anti-Aβ antibody improves LTP in hippocampal slices

In addition to the behavioral and histological effects of 10D5, we wanted to determine whether it affected electrophysiological parameters. At the end of water maze testing, brains were removed from WT and PDAPP mice, and LTP was assessed in the CA1 region of the hippocampus. Theta burst stimulation successfully induced LTP in slices from WT mice (EPSP increase; 62 ± 15%; $n = 6$). As reported previously (Chapman et al., 1999), the theta burst stimulation failed to induce LTP in untreated PDAPP mice (14 ± 10%; $n = 8$; $p < 0.02$ by *t* test). In slices from PDAPP plus 10D5 mice, however, LTP was consistently induced (61 ± 24%; $n = 6$) and did not appear to differ from wild-type levels (Fig. 7*A*,*B*). Together with the behavioral data, these electrophysiological data suggest that Aβ is contributing to functional abnormalities in the PDAPP mice.

Discussion

PDAPP mice on a C57BL/6 genetic background demonstrated both age-independent and age-dependent spatial learning deficits in the water maze. The age-related decline in spatial learning was significantly more severe than the subtle decline observed in wild-type littermate mice. The fact that the age-related decline in water maze performance occurred only after substantial Aβ deposition suggests that something directly or indirectly linked with the process of Aβ aggregation is responsible for this decline. The fact that the parenteral administration of the anti-Aβ antibody 10D5 to old mice (17–19 months of age) significantly improved performance in the water maze, decreased Aβ deposition, increased plasma Aβ, and improved LTP strongly suggests that Aβ accumulation is responsible for the age-related decline in performance in PDAPP mice.

Our study is the first to characterize spatial learning in PDAPP mice on a pure genetic background (C57BL/6), despite the fact that this strain is recommended for water maze testing (Crawley, 2000). We also found impaired learning in PDAPP mice, relative to wild-type littermate controls, at 4–5 months of age, several months before Aβ begins to aggregate in the brain. It seems likely that this early deficit in spatial learning is secondary to overex-

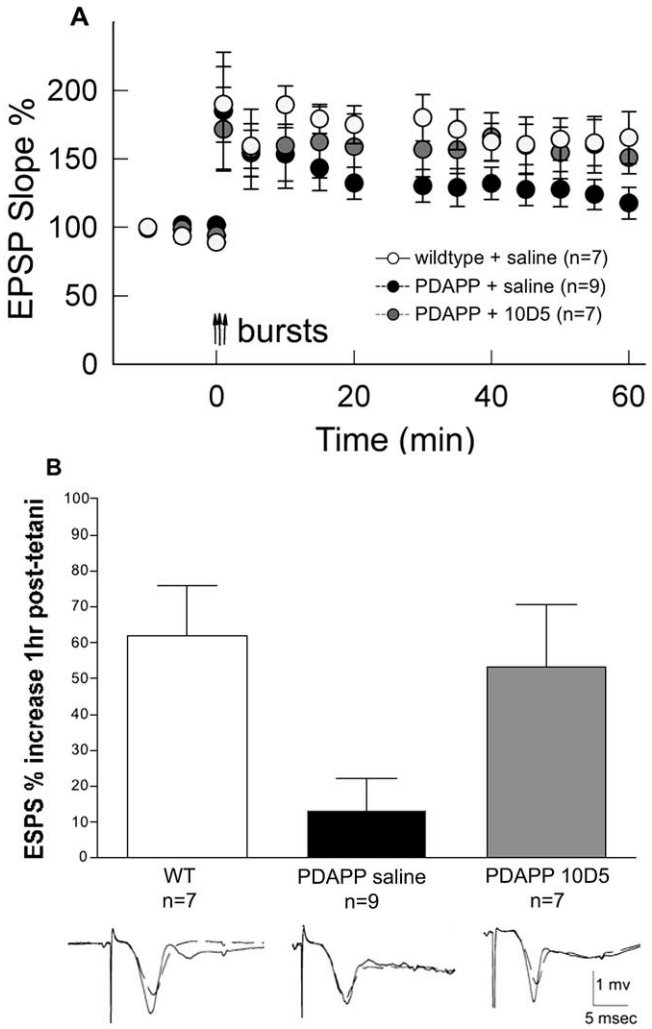

Figure 7. Treatment with 10D5 improved hippocampal LTP in aged PDAPP mice. ***A***, Six series of tetanic bursts consisting of six trains of six pulses were delivered at time 0 (arrows). At 60 min after tetani, PDAPP plus saline mice exhibited impaired LTP compared with PDAPP plus 10D5 and WT mice. ***B***, The bars represent levels of LTP 60 min after the induction of tetani. Traces depict EPSPs before (dotted traces) and 60 min after (solid traces) tetanic bursts. Error bars represent SEM.

pression of high levels of human APP with the V717F familial AD mutation or APP fragments and less likely secondary to Aβ. First, several other APP transgenic mice that have been developed have similar or even higher levels of soluble Aβ early in life (Fryer et al., 2003), yet show no learning deficits until after the onset of Aβ aggregation (Pompl et al., 1999; Westerman et al., 2002). Second, overexpression of APP, in the absence of Aβ aggregation, has resulted in spatial learning abnormalities in mice (Hsiao et al., 1995; Moechars et al., 1999; Kumar-Singh et al., 2000). Because APP has been shown to be neurotrophic under certain conditions (Mucke et al., 1996), it is possible that expression of high APP levels throughout development may alter cell number and connectivity via deleterious effects on apoptosis or other normal developmental processes. Indeed, PDAPP mice also exhibit a number of other abnormalities, including a smaller corpus callosum, fornix commissure, and hippocampus (Dodart et al., 2000; Gonzalez-Lima et al., 2001), increased synaptic density (Dodart et al., 2000), CA1 dendritic spine loss (Lanz et al., 2003), lower core body temperature, altered circadian rhythm (Huitron-Resendiz et al., 2002), and abnormal hippocampal LTP (Larson et al., 1999; Giacchino et al., 2000), suggesting that overexpression of APP or Aβ may affect brain development. The presence of the V717F mutation in APP also results in altered cleavage of APP at the γ-secretase site, which, in addition to increasing levels of $A\beta_{42}$, is also likely to result in increased levels of γ-cleaved C-terminal APP fragments. Because the C-terminal domain of APP can act as a transcription factor in the nucleus (Cao and Sudhof, 2001, 2004), this suggests another way in which APP overexpression could alter neuronal function and behavior. Third, in humans, Aβ aggregation and build-up in Down syndrome and in late-onset AD begin ~10–20 years before even the earliest evidence of any cognitive decline (Scott et al., 1983; Albert, 1992; Goldman and Morris, 2001; Morris and Price, 2001). Thus, initial Aβ aggregation in Down syndrome and AD appears to lead to downstream secondary events and dementia, but this takes many years to develop in humans and is not present before Aβ deposition.

Chen et al. (2000) showed that PDAPP mice on a mixed background also exhibit age-related spatial learning deficits in the water maze. In their task, the position of the escape platform was changed after a mouse demonstrated (by an escape-latency-based criterion) that it had learned the position. The key feature of the task was that the mice had to continuously relearn the position of the platform. In the current study, PDAPP mice on a C57BL/6 background also had to continuously relearn the position of the platform. One difference between these studies is that on the task used by Chen et al. (2000), each mouse supposedly learned the platform position to an approximately equal degree before the position was changed. In the task that we used, all mice were subjected to an equal number of trials (20) before the position was changed. Another difference is that in this study, both wild-type and PDAPP mice swam faster as they aged, making an escape latency-based criterion inappropriate. However, using swim distance as the dependent variable negated that confound and allowed for the observation that old PDAPP mice showed almost no evidence of learning when given 20 trials over 5 d to learn the position of the escape platform. Young and middle-aged PDAPP mice, although they performed worse than even old wild-type mice, showed at least some evidence of improved spatial navigation over the course of 5 d. Thus, the task used in this study proved to be too difficult for old, but not young or middle-aged, PDAPP mice. Although the performance of old wild-type mice showed some decline with age, the age-related decline in the performance of old PDAPP mice was much more dramatic, suggesting that the water maze task used in this study allowed us to demonstrate an important and significant age-by-genotype interaction.

The use of the anti-Aβ antibody 10D5 provided an interesting test of a possible treatment strategy, as well as useful data regarding the mechanism of the cognitive decline. PDAPP mice treated with 10D5 performed significantly better than saline-treated littermate PDAPP mice in the water maze spatial navigation task. After 8–12 weekly injections, hippocampal Aβ and fibrillar Aβ loads were decreased by >50%, and carbonate-soluble and -insoluble $A\beta_{40}$ and $A\beta_{42}$ were lowered, although not significantly. Furthermore, hippocampal synaptic efficacy in an LTP paradigm was deficient in untreated, but not 10D5-treated, PDAPP mice. The 10D5-treated PDAPP mice in this study never attained wild-type-like levels of spatial navigation performance, suggesting that the age-dependent, but not the age-independent, learning deficit was ameliorated. The observation that hippocampal LTP is fully restored in 10D5-treated PDAPP mice, but that learning performance is only partially rescued, further sug-

gests that the early learning deficit is a result of other, perhaps developmental, factors.

Other studies have shown effects of immunotherapeutic treatments targeting Aβ. For instance, active immunization with Aβ can both reduce Aβ deposition (Schenk et al., 1999) and improve spatial learning deficits in APP transgenic mice (Janus et al., 2000; Morgan et al., 2000; Arendash et al., 2001). Others have reported improvements in learning ability after passive treatment of APP transgenic mice with monoclonal Aβ antibodies. For example, Dodart et al. (2002) treated 11-month-old PDAPP mice acutely with the anti-Aβ antibody m266 and found reduced object recognition and hole-board spatial learning deficits after only 24–72 h in the absence of any observable effect on Aβ deposition. Kotilinek et al. (2002) treated 9- to 11-month old Tg2576 mice with the anti-Aβ monoclonal antibody BAM10 over several days and found a partial reduction in water maze learning deficits but no reduction in Aβ deposition levels, and Wilcock et al. (2004) reported improved Y-maze alternation performance and reduced Aβ deposition in 22-month-old Tg2576 mice after 3 months of treatment with the anti-Aβ antibody 2286. The current study expands on those previous reports of passive immunization by demonstrating the effects of systemic injection of anti-Aβ antibody in very old PDAPP mice on behavior (improved spatial navigation), neuropathology (reduction of diffuse and fibrillar plaque load by >50%), and hippocampal electrophysiology (amelioration of LTP deficits).

These effects of 10D5 treatment provide direct evidence for the detrimental effects of Aβ aggregation and deposition. The effect on spatial navigation was evident by the first several days of testing and after only two weekly injections. To our knowledge, this is the first study to demonstrate both reduced Aβ deposition and improved spatial navigation performance in the water maze in an APP transgenic mouse after treatment with a monoclonal anti-Aβ antibody (passive immunization). Additionally, this study is the first to demonstrate improvement of hippocampal LTP in treated animals, suggesting that Aβ somehow disrupts hippocampal function and learning ability. The mechanism of Aβ reduction in the brain after peripheral monoclonal antibody treatment in this study remains to be determined. Possibilities include direct binding to Aβ plaques in the brain followed by microglial activation (Wilcock et al., 2004), direct binding and disruption of plaques (Bacskai et al., 2002), binding to soluble Aβ in the brain (Dodart et al., 2002), and/or a peripheral "soluble Aβ sink" mechanism (DeMattos et al., 2001; Lemere et al., 2003). Additional studies are required to sort out the mechanism(s) of the effects of each of the different anti-Aβ antibodies. To summarize, this and other studies on the effects of passive immunization with anti-Aβ antibodies support the idea that Aβ represents a viable target in the treatment of Alzheimer's disease and that this treatment strategy has a chance to be effective and safe. Thus, cautiously proceeding ahead with trials of passive immunization targeting Aβ in humans appears appropriate at this time.

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