

# Attenuated Hippocampus-Dependent Learning and Memory Decline in Transgenic TgAPP<sup>swe</sup> Fischer-344 Rats

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Alzheimer's disease (AD) is characterized by increased  $\beta$  amyloid (A $\beta$ ) levels, extracellular A $\beta$  deposits in senile plaques, neurofibrillary tangles, and neuronal loss. However, the physiological role of normal levels of A $\beta$  and its parent protein, the amyloid precursor protein (APP) are unknown. Here we report that low-level transgenic (Tg) expression of the Swedish APP mutant gene (APP<sup>swe</sup>) in Fischer-344 rats results in attenuated age-dependent cognitive performance decline in 2 hippocampus-dependent learning and memory tasks compared with age-matched nontransgenic Fischer-344 controls. TgAPP<sup>swe</sup> rats exhibit mild increases in brain APP mRNA (56.8%), A $\beta$ -42 (21%), and A $\beta$ -40 (6.1%) peptide levels at 12 mo of age, with no extracellular A $\beta$  deposits or senile plaques at 6, 12, and 18 mo of age, whereas 3- to 6-fold increases in A $\beta$  levels are detected in plaque-positive human AD patients and transgenic mouse models. The data support the hypothesis that a threshold paradigm underlies A $\beta$ -related pathology, below which APP expression may play a physiological role in specific hippocampus-dependent tasks, most likely related to its neurotrophic role.

## INTRODUCTION

Alzheimer's disease (AD) is characterized by the progressive loss of cognitive abilities associated with increased brain  $\beta$  amyloid (A $\beta$ ) levels, extracellular A $\beta$  deposits in senile plaques, intraneuronal neurofibrillary tangles, and neuronal death (1). Although a seemingly straightforward pathological definition, clinical and genetic heterogeneity depict complex neurological disease paradigms. The central pathogenic hypothesis of AD focuses on increased A $\beta$  production, however, it is still unknown as to how A $\beta$  causes AD on one hand, and on the other hand, what normal physiological role A $\beta$  performs because A $\beta$  is detected in healthy individuals throughout life (2,3). The A $\beta$  hypothesis is supported by the linkage of amyloid precursor protein (APP) variants to human familial AD (4). Although accounting for less than 1% of AD cases, study of these APP variants comprise a focus for analysis of disease mechanisms. One AD gene, the double mutant (K670N, M671L) APP found in 1 form of Swedish familial AD (APP<sup>swe</sup>) (5) results in 3- to 6-fold elevation of A $\beta$  production. AD is then characterized as autosomal dominant with high penetrance, albeit variable clinical symptoms, and onset averaging around 50 y of age (6).

Disease complexities are exposed in transgenic modeling designed to recapitulate APP<sup>swe</sup> AD in animal models. In the Tg2576 mouse model (7), human prion protein-directed APP<sup>swe</sup> transgenic expression results in 5-fold elevation of A $\beta$ 40 and 14-fold elevation of A $\beta$ 42 associated with senile plaques at 11 to 13 mo, and impaired hippocampus-dependent performance in the Morris water maze. However, neuronal loss and neurofibrillary

tangles were not detected. Additionally, despite concomitant widespread amyloid plaques in amygdala, Tg2576 mice did not show impaired amygdala- and hippocampus-dependent Pavlovian fear-conditioning task (8). In another transgenic APP<sup>swe</sup> model, TgAPP23 (9), murine thy-1 directed APP<sup>swe</sup> transgenic expression results in 5-fold elevation of brain A $\beta$ -42 peptide levels and A $\beta$  plaques in 6-mo-old mice. However, A $\beta$  plaque formation detected at 6 mo is preceded by impaired hippocampus-dependent performance in the Morris water maze at 3 mo (10).

We therefore investigated whether transgenic expression of APP<sup>swe</sup> in inbred Fischer-344 rats would recapitulate the full spectrum phenotype of human APP<sup>swe</sup> AD. Because the inbred Fischer-344 rat strain is a well-characterized experimental model of human age-related memory disorder (11), we hypothesized that its genetic susceptibility to hippocampus-dependent spatial learning and memory decline as measured in the Morris water maze would provide a conducive genetic background. We developed a transgenic Fischer rat model, TgAPP<sup>swe</sup>, that expresses the APP<sup>swe</sup> mutation driven by the platelet-derived growth factor (PDGF) promoter. To our surprise, the cognitive performance of TgAPP<sup>swe</sup> rats was significantly better than age-matched 6- and 12-mo-old control Fischer-344 rats respectively in 2 hippocampus-dependent learning and memory tasks. In contrast to transgenic APP<sup>swe</sup> mouse models (7), the increases in brain APP mRNA and peptide, as well as A $\beta$  peptide levels in TgAPP<sup>swe</sup> rats, are much less and do not result in extracellular A $\beta$  deposits. These data suggest that levels of APP or A $\beta$  that do not result in extracellular amyloid deposits may play a role in hippocampus-dependent tasks.

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## MATERIAL AND METHODS

### Transgenic Rat Development

All animal experimentation was conducted in accordance with protocols approved by the Boston University Institutional Animal Care and Use Committee. Fischer-344 rats were purchased from Harlan Inc (Indianapolis, IN, USA). The PDGF-hAPP<sup>swe</sup> transgene was synthesized and tested in KSK's lab. A 4.36-Kb *SpeI/HindIII* fragment containing the transgene was gel-purified and microinjected into Fischer-344 rat one-celled embryos as described (12). Transgenic founders and transgenic offspring were identified by Southern blot analysis as described (12). Four founders were produced and 1 transgenic line (line 17, the line expressing the highest level of hAPP<sup>swe</sup>) was bred to homozygosity. All experiments were performed with homozygous TgAPP<sup>swe</sup> male rats originated from line 17.

### Study Cohorts

Six-month-old Fischer-344 male rats ( $n = 13$ ), 6-mo-old TgAPP<sup>swe</sup> male rats ( $n = 13$ ), 12-mo-old Fischer-344 male rats ( $n = 10$ ), and 12-mo-old TgAPP<sup>swe</sup> male rats ( $n = 10$ ) were tested in this study. The subjects were individually housed in standard 27 × 48 × 20-cm plastic cages (Allentown Caging Equipment, Allentown, NJ, USA) from 3 mo of age. All were maintained on LabDiet 5001 rodent chow (Harlan Teklad, Madison, WI, USA). The food pellets and water were made available ad lib.

### Social Recognition Task

All tests were conducted in dim red light during the dark phase of a 12 : 12 h light/dark cycle and after a 2-h acclimation period. All observations were recorded onto videotape using a video camera equipped with an infrared light source. Eight-week-old male Sprague Dawley rats (Harlan Inc, Indianapolis, IN, USA) were used as social stimuli. Testing began when a juvenile was introduced in the subject's home cage for a 5-min initial exposure. At the end of the trial, the juvenile was removed and returned to its cage. A 2nd 5-min exposure to the same juvenile was conducted after a certain interval of delay. The delay intervals used in this experiment were 5 min and 30 min. Social investigatory behavior was defined as described (13,14) and included direct contact while sniffing, following closely, nosing, pawing, grooming, or generally inspecting any body surface of the juvenile as well as the tip of the subject's nose being proximally oriented to within approximately 1 cm of the juvenile. Observational data were scored and analyzed using The Observer Base Package for Windows Version 3.0 (Noldus Information Technology, Wageningen, The Netherlands). Only the 1st min was used in the analysis.

### Assessment of Inherited Food Preference

Four-month-old Fischer-344 male rats ( $n = 8$ ) purchased from Harlan Inc were used in this experiment. Preshaped subjects were 1st deprived of food for 22 h. Two flavored foods in separate jars (spaced 10 cm apart) were then presented in the subject's home cage. The scented foods were removed after 2 h and consumption was measured. Water was absent during each 2 h session. This

procedure was repeated on subsequent consecutive day with 1 flavored food pair per day. The flavored food pairs tested were cinnamon against cocoa, clove against garlic, marjoram against thyme, ground anise against cumin, curry against ginger, sage against onion, and turmeric against nutmeg. We allowed the subjects to consume 3 rodent chow pellets immediately following each 2-h test. Subjects were presented with each type of scented food only once throughout the test.

### Social Transmission of Food Preference (STFP) Task

All tests were conducted in the light phase of a 12 : 12 light/dark cycle. Powdered food was presented in 16 oz powdered food feeders and jar holders designed to minimize spillage (Allentown Caging Equipment). Flavored foods were prepared fresh on the day of the test by mixing LabDiet 5001 rodent meal with, respectively, 1% (w/w) cinnamon, 2% (w/w) cocoa, 0.25% (w/w) clove, 0.2% (w/w) garlic, 2% (w/w) marjoram, 1% (w/w) thyme, 1% (w/w) ground anise, 0.4% (w/w) cumin, 0.25% (w/w) curry, 0.25% (w/w) ginger, 0.25% (w/w) sage, 0.25% (w/w) onion, 0.75% (w/w) turmeric, and 0.5% (w/w) nutmeg. The STFP task was performed essentially as described (15). Subjects were 1st trained to consume powdered food (LabDiet 5001 rodent meal) for 5 d prior to testing. During this "shaping" period, subjects were placed on a 23.5-h food-deprivation schedule and offered powdered food in their home cages for 30 min/d for 3 consecutive days. We allowed the subjects to consume 3 rodent chow pellets immediately following the 1st and 2nd 30-min session. After the 3rd 30-min session, powdered food was left in the subject's home cage for 2 additional days. Food consumption was measured only during the 1st 3 d of "shaping." A subject was considered "shaped" when it consumed at least 1 g of powdered food in 2 consecutive 30-min sessions. Subjects that did not meet this criterion were excluded from the test. The Fischer-344 male rats and TgAPP<sup>swe</sup> male rats were used as observers in this experiment. Eight-week-old male Sprague Dawley rat juveniles (purchased from Harlan Inc) were used as demonstrators. Preshaped observers were 1st deprived of food for 22 h. To minimize aggressive behavior, demonstrators were anesthetized and powdered at the head end with a specific scented food as described (16,17). The demonstrator was immediately placed in the observer's home cage, and the observer was allowed to explore the juvenile for 20 min. At the end of this exploratory period, the demonstrator was removed and a 5-min or 3-h time delay was enforced. At the end of the time delay, the observer was offered 2 scented foods of a predetermined odor pairing in separate jars (10 cm apart), one of which was identical with the scented food presented by the demonstrator. The food was presented in a counterbalanced manner in the absence of water. The flavored foods were removed after 2 h and consumption was measured. The pre-assigned odor pairings were clove against garlic for the 5-min retention time and curry against ginger for the 3-h retention time. The ratio of the consumed "trained odor" over the total weight of food eaten was used to calculate percent trained-odor preference.

### Morris Water Maze Task

The Morris water maze system (San Diego Instruments, San Diego, CA, USA) consisted of a circular pool (1.83 m in diameter

and 0.95 m deep) and a movable square platform (13 cm × 13 cm and 31 cm in high). The pool was filled with water (25 ± 1 °C) and rendered opaque by the addition of 0.5 gallons of 2% reduced fat milk. The pool was used together with the SMART computer tracking system (San Diego Instruments) that monitored and recorded the rats' swim path for later analysis. The water maze was divided into 4 imaginary quadrants.

**Hidden platform version.** The water maze system was located in a small observation room with numerous salient visual cues in predefined locations in the vicinity (1.2 to 2.4 m) of the pool. Testing was performed in 2 consecutive days. Briefly, the pool was filled until the platform was submerged 1 cm below the surface of the water. A trial was initiated by placing the rat in the water facing the pool wall in 1 of the chosen quadrants. Twelve trials or swims were given per day from 1 of 3 randomized start positions, which were located adjacent to the wall in the center of the 3 quadrants that did not contain the platform. Animals were tested counterbalanced with respect to subject groups. Latency to escape from the water was recorded and a 60-s maximum swim time was imposed on each trial. Rats were guided to the escape platform if they failed to locate it in the specified time. After each trial, a 35-s interval was imposed while the rat was on the platform. At the end of the 24th trial, the platform was removed and the rat was released into the quadrant diagonally opposite to that which contained the platform. During this probe trial, the rat was allowed to search for 1 min.

**Visible platform version.** This experiment was performed 24 h following the completion of the probe trial. This time, all visual cues were removed from the room and the platform was raised 2 cm above the surface of the water. The platform was cued by attaching two 15 cm high dark cylinders onto it. For each of 12 trials, the rat was released in a random fashion into each of 4 quadrants to locate the visible platform and escape the water. The platform was likewise moved in a random fashion for each trial. Rats were tested counterbalanced with respect to subject groups.

### Protein Blot Analysis

At 6 mo, brains were isolated after phosphate-buffered saline perfusion from heterozygous TgAPPswe transgenic male rats. Whole brain protein extracts were analyzed essentially as described (18) using an APP695 stably transfected CHO cell line protein extract as positive control.

### RNA Blot Analysis

After completion of behavioral analysis at 12 mo, brains were isolated from TgAPPswe and control rats after phosphate-buffered saline perfusion and quickly frozen ( $n = 6$ ). Total cellular RNAs were isolated from hemi-brains, and 5 µg was separated by size onto 0.8% formaldehyde agarose gel to distinguish endogenous rat APP from slightly shorter transgenic APPswe mRNA. The APP transgene was used as probe. Quantitation was performed by densitometric analysis of autoradiograms.

### Aβ-Peptide Analysis

After behavioral analysis, brains were isolated from 12-mo-old TgAPPswe and non-transgenic control rats ( $n = 6$ ). Half of the

brains were assigned to analysis of Aβ-peptide levels, and half designated for histological analysis. Aβ-peptide levels were done essentially as described (19).

### Histology

Analysis of serial brain sections (6 µ) was done using PAS-Bielschowsky and Congo-red staining, and immunostaining with anti-Aβ-peptide antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA).

### Statistical Analyses

Data from behavioral experiments were analyzed by two-way repeated measure analysis of variance (ANOVA), one-way ANOVA, and *t*-tests when appropriate using the SigmaStat software, version 2.0 for Windows (SPSS, Chicago, IL, USA). Comparative analysis of brain Aβ peptide concentrations was done by one-way ANOVA using the same software. All statistical tests were two-tailed and in all studies, differences were considered significant at the  $P < 0.05$  level.

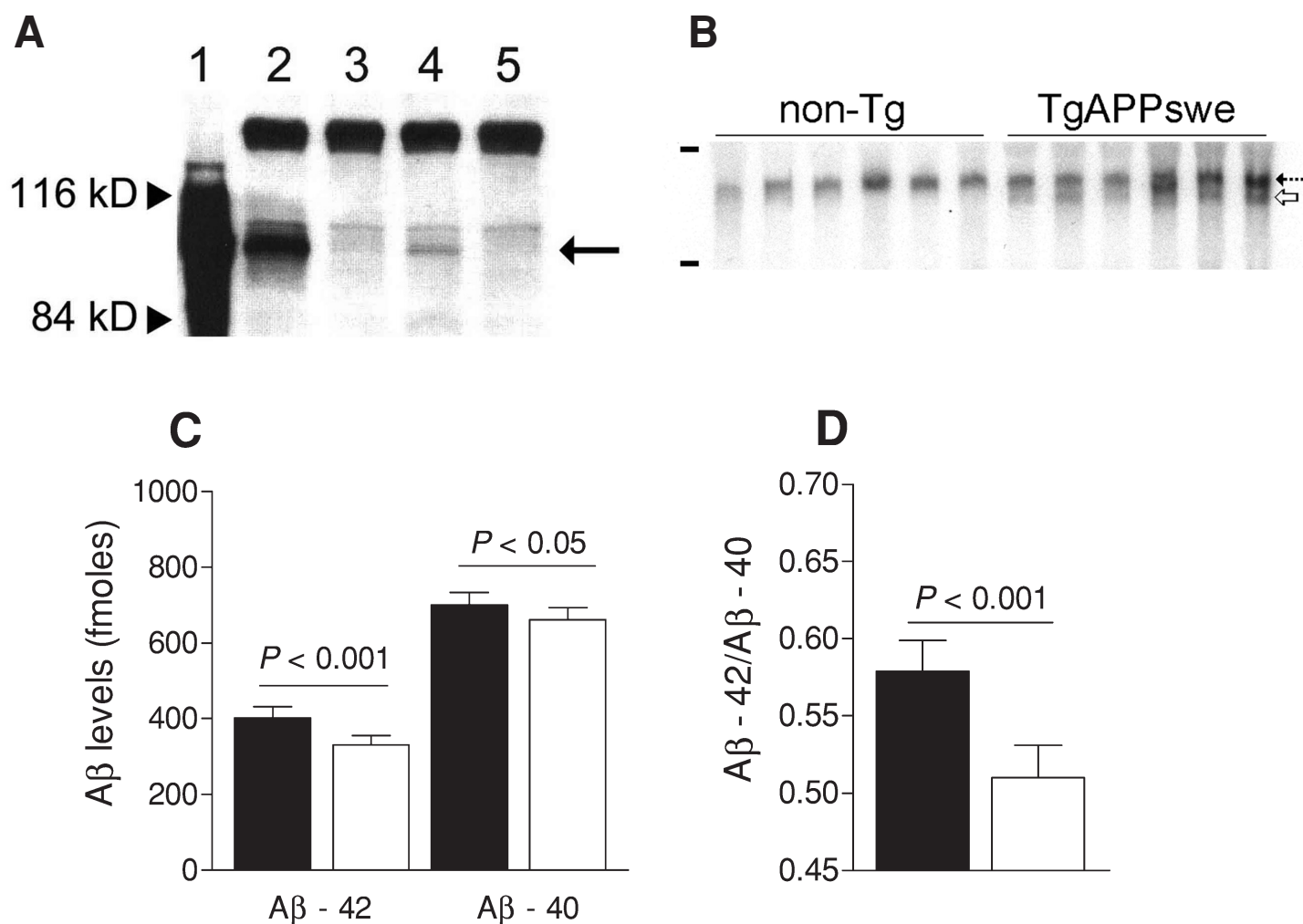
## RESULTS

Transgenic TgAPPswe inbred Fischer-344 rats were developed using a minigene construct containing human APPswe cDNA driven by the PDGF promoter. The PDGF promoter was previously used in a transgenic M146L presenilin1 variant AD mouse model (20). Southern blot analysis identified 4 transgenic founders, 2 of which produced transgenic lines (data not shown). Protein blot analysis confirmed human APPswe expression in the 2 transgenic lines, compared with nondetection of human APP in nontransgenic controls (Figure 1A). The high expresser transgenic line was bred to homozygosity and used for study. Nontransgenic controls comprised inbred age-matched Fischer-344 rats.

Analysis of hemi-brain total cellular RNA from homozygous 12-mo-old TgAPPswe male rats revealed a 56.8% increase in APP mRNA levels compared with age-matched control Fischer rats ( $P < 0.0001$ ) (Figure 1B). Analysis of hemi-brain protein extracts of homozygous 12-mo-old TgAPPswe male rats revealed a 21% increase in Aβ-42 peptide levels compared with age-matched control Fischer rats ( $P < 0.001$ ), a 6.1% increase in Aβ-40 levels ( $P < 0.05$ ), and a 13.5% increased Aβ42/Aβ40 ratio ( $P < 0.001$ ) (see Figure 1C and 1D). Multiple histological examinations (immunochemical, PAS-Bielschowsky, and Congo-red staining) of serial brain sections of both TgAPPswe and control Fischer brain sections at 6, 12, and 18 mo showed no evidence of extracellular amyloid deposits or plaques (data not shown).

To investigate the effects of APPswe on cognitive function in TgAPPswe rats, we measured performance of TgAPPswe and control Fischer-344 rats in both hippocampus-dependent and hippocampus-independent learning and memory tasks. Experiments were performed on 2 independent groups of naive male subjects at 6 and 12 mo of age.

To measure hippocampus-dependent learning and memory, subjects were tested 1st on a socially induced diet choice paradigm (STFP)—a natural and nonspatial learning and memory task that involves the development of preference for a particular scented

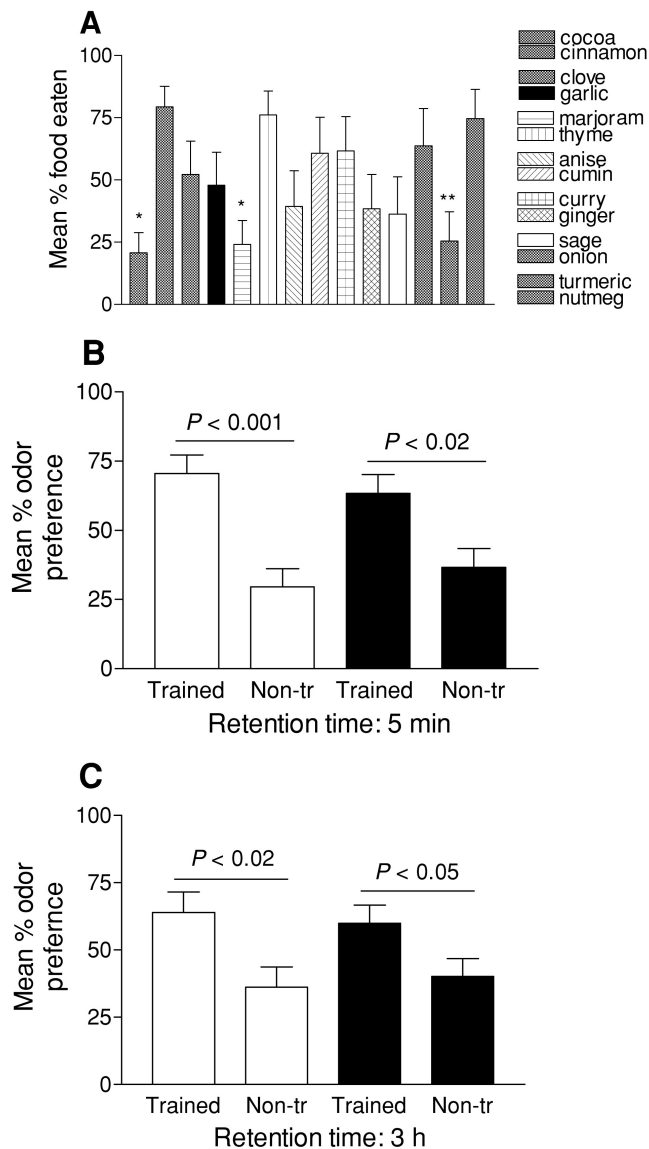


**Figure 1.** Biochemical characterization of TgAPPswe rats. A: Western blot analysis of brain protein extracts (at 6 mo of age) from transgenic (Lane 2, TgAPPswe line 17 utilized in this study; Lane 4, another TgAPPswe line but with lower expression) and control nontransgenic (Lanes 3 and 5) inbred Fischer-344 rats. Lane 1 corresponds to APP695 stably transfected CHO cell line extracts that serve as positive control. The hAPP polypeptide is marked (→). B: RNA blot analysis of 12-mo-old TgAPPswe compared with control nontransgenic Fischer rats (non-Tg) revealed 56.8% increase of total APP mRNA levels in TgAPPswe rat samples. Endogenous rat APP (←); transgenic human APPswe (⇐). C and D: Aβ peptide levels in TgAPPswe ( $n = 6$ ) and control nontransgenic ( $n = 6$ ) inbred Fischer-344 rats at 12 mo of age. ■, TgAPPswe; □, control nontransgenic Fischer.

food after an “observer” rat interacts with a “demonstrator” rat that has recently eaten a distinctively scented food (15,21). The mechanism underlying this learning has been shown to involve an association between 2 odors present in the “observer” rat’s breath, the odor of the recently eaten food and carbon disulfide, a natural constituent of rat’s breath (22). As a 1st step, we performed a control experiment measuring the inherent preference for each odor within a pair considered for STFP testing in Fischer-344 male rats, to assess potential genetic predisposition for specific scented foods that could confound subsequent STFP tests. This pretest is valid for both TgAPPswe and non-transgenic control rats because both are in the inbred Fischer-344 genetic background. As shown in Figure 2A, Fischer rats demonstrated inherent preference for cinnamon over cocoa ( $F_{(1,14)} = 25.825$ ,  $P < 0.001$ ); thyme over marjoram ( $F_{(1,14)} = 14.382$ ,  $P = 0.002$ ); and nutmeg over turmeric ( $F_{(1,14)} = 8.722$ ,  $P = 0.01$ ). These pairings were excluded from STFP experi-

ments performed on control and transgenic subjects. At 6 mo of age, both control Fischer and TgAPPswe rats demonstrated efficient performance in this behavioral task. At 5 min retention time, both groups had equivalent, strong preference for the trained odor (see Figure 2B, control Fischer:  $F_{(1,20)} = 18.458$ ,  $P < 0.001$ ; TgAPPswe:  $F_{(1,20)} = 7.754$ ,  $P < 0.02$ ). Similar results were observed at 3 h retention time (see Figure 2C, control Fischer:  $F_{(1,20)} = 6.744$ ,  $P < 0.02$ ; TgAPPswe:  $F_{(1,20)} = 4.372$ ,  $P < 0.05$ ).

To further test potential hippocampus-dependent cognitive performance differences between control Fischer and TgAPPswe rats, we performed a classical test of spatial learning and memory, the Morris water maze (MWM) task (23–25) wherein the subject must learn multiple spatial relationships between extra-maze cues and the relationships of distal cues to the platform to find the “hidden escape platform” in a large water tank (25). At 6 mo of age, control Fischer rats exhibit an impairment of acquisition per-



**Figure 2.** Social transmission of food preference in 6-mo-old Fischer and TgAPPsw rats. A: Mean percent ( $\pm$ SEM) of food eaten in a choice test (to assess inherited food preference) by 4-mo-old Fischer male rats presented with the indicated scented food pairings. (\* $P < 0.003$ , \*\* $P = 0.01$ ). Mean percent odor preference ( $\pm$ SEM) in 6-mo-old Fischer ( $\square$ ) and TgAPPsw ( $\blacksquare$ ) observers at 5 min (B) and 3 h (C) retention times. Non-tr, non-trained odor.

formance in the task compared with TgAPPsw rats (Figure 3A,  $F_{(1,84)} = 4.972$ ,  $P = 0.046$ ). On the probe trial, both groups show equivalent target selectivity demonstrating preference for the target quadrant over all the remaining quadrants (see Figure 3B, Fischer:  $P < 0.001$ ; Figure 3C, TgAPPsw:  $P < 0.001$ , Tukey's pairwise multiple comparison test comparing percent search in target quadrant against opposite, target quadrant against right, and target quadrant against left following one-way ANOVA). However, examination of latency to cross the training site the 1st time during the probe trial—another discriminatory measurement of

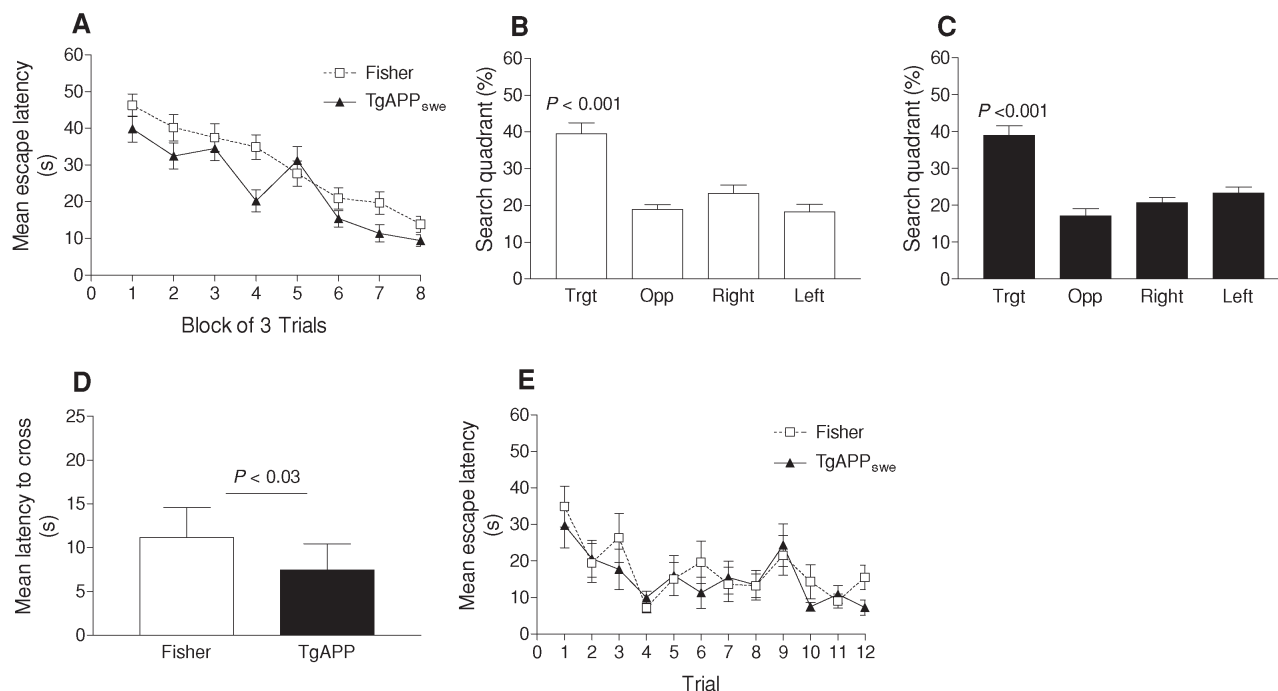
search accuracy for the hidden platform (26)—showed that the TgAPPsw rats were able to locate the hidden platform more quickly than control Fischer rats (see Figure 3D, Fischer mean latency =  $11.185 \pm 3.42$  s; TgAPPsw mean latency =  $7.477 \pm 2.96$  s,  $H = 5.109$ ,  $P < 0.03$ , one-way ANOVA on ranks), indicating better navigational performance of TgAPPsw rats. Both groups had comparable average swim speeds (swim speed Fischer =  $14.92 \pm 0.48$  cm/s; swim speed TgAPPsw =  $15.22 \pm 0.51$  cm/s,  $F_{(1,24)} = 0.188$ ,  $P = 0.669$  in probe trial) and were equally efficient in localizing the escape platform in a visible platform version of the MWM (see Figure 3E,  $F_{(1,131)} = 0.308$ ,  $P = 0.589$ ). These results rule out the possibility that a motor-sensory difference between the groups could explain the differential performance of 6-mo-old control Fischer and TgAPPsw rats in the MWM task.

To test for hippocampus-independent, vomeronasal-dependent learning and memory, both TgAPPsw and age-matched control Fischer rats were challenged with a social recognition (SR) task wherein familiarity-dependent variations in duration of social investigation of conspecifics, presented at different intervals (retention times), are used to assess social recognition (13,14,27,28).

On exposure to a conspecific, rats display bouts of olfactory investigation, the duration of which depends upon familiarity with the conspecific. Unfamiliar conspecifics are explored longer and more intensively than familiar ones. At 6 mo of age, both TgAPPsw and control Fischer rats showed strong social recognition performance at 5 min (Figure 4A; TgAPPsw:  $F_{(1,12)} = 81.327$ ,  $P < 0.001$ ; control Fischer:  $F_{(1,12)} = 93.979$ ,  $P < 0.001$ , one-way repeated measures ANOVA) and 30 min (see Figure 4B; TgAPPsw:  $F_{(1,12)} = 11.690$ ,  $P < 0.003$ ; control Fischer:  $F_{(1,12)} = 6.151$ ,  $P = 0.02$ , one-way repeated measures ANOVA) retention times.

The unexpected result of improved navigational performance of TgAPPsw rats compared with age-matched control Fischer rats in hippocampus-dependent MWM task prompted us to further investigate these observations. We performed the same behavioral tests on a new group of naive male subjects at 12 mo of age to determine if cognitive performance differences would be sustained and/or altered by age. In the SR test, both groups perform equivalently, reproducing SR results at 6 mo of age. Both groups were able to recognize conspecifics at 5 min (Figure 5A, Fischer:  $F_{(1,9)} = 19.399$ ,  $P = 0.002$ ; TgAPPsw:  $F_{(1,9)} = 49.900$ ,  $P < 0.001$ , one-way repeated measures ANOVA) and 30 min (see Figure 5B, Fischer:  $F_{(1,9)} = 14.523$ ,  $P = 0.004$ ; TgAPPsw:  $F_{(1,9)} = 18.900$ ,  $P < 0.002$ , one-way repeated measures ANOVA) retention times. In contrast, a sharp decline in performance was detected in control Fischer rats in the STFP paradigm (Figure 6). Although both groups demonstrated significant preference for the trained odor at a 5-min retention time (see Figure 6A, Fischer:  $F_{(1,18)} = 25.593$ ,  $P < 0.001$ ; TgAPPsw:  $F_{(1,18)} = 58.347$ ,  $P < 0.001$ ), the control Fischer group failed to discriminate the trained from the non-trained odor at 3 h retention time (see Figure 6B,  $F_{(1,18)} = 1.355$ ,  $P = 0.26$ ). In contrast, the TgAPPsw group showed significant preference for the trained odor after 3 h retention time (see Figure 6B,  $F_{(1,18)} = 8.777$ ,  $P < 0.01$ ).

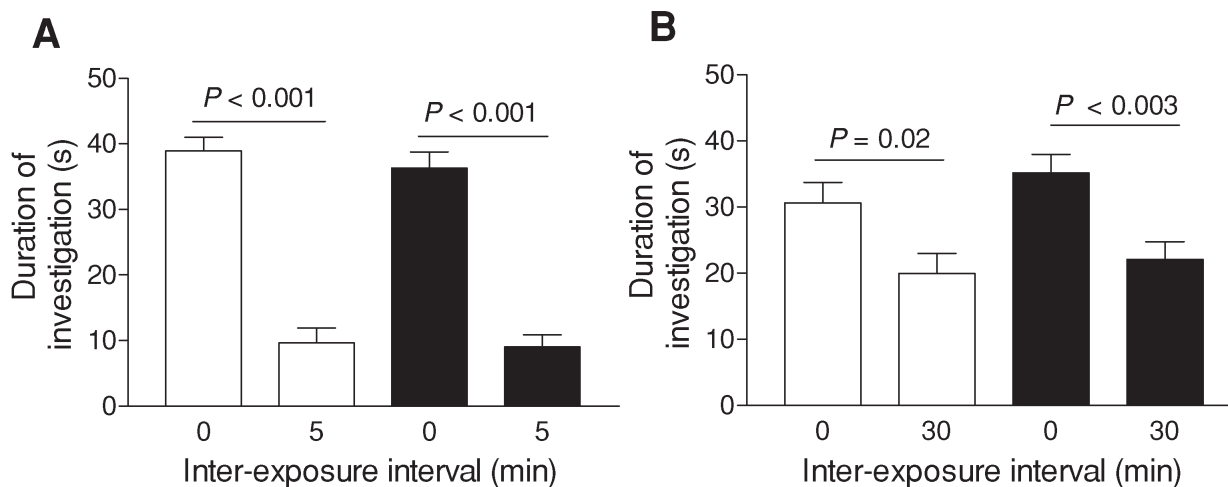
The MWM task further corroborates cognitive performance differences between control Fischer and TgAPPsw rats at 12 mo of



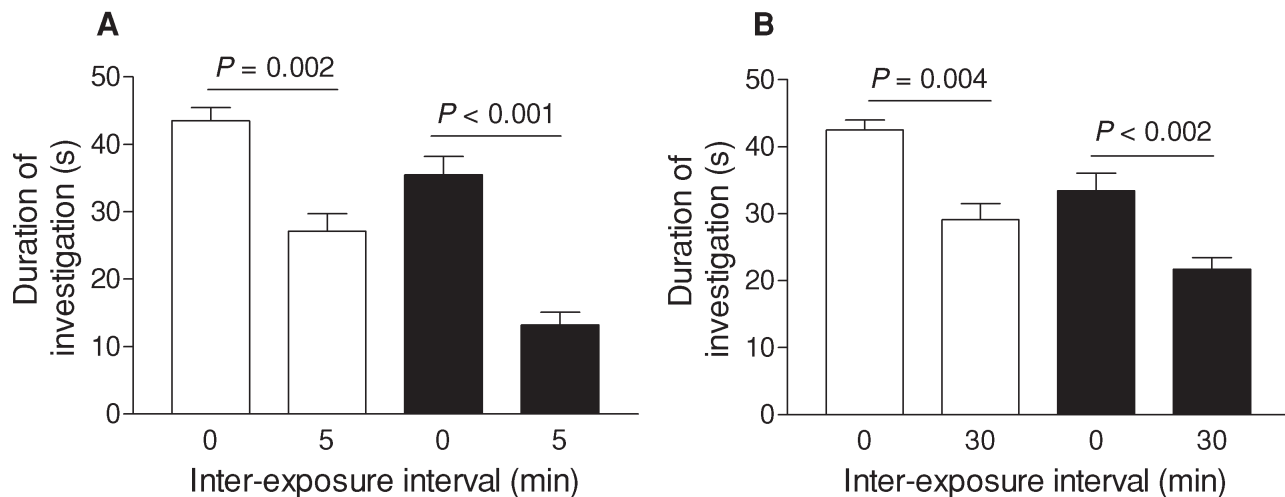
**Figure 3.** Spatial learning in 6-mo-old Fischer and TgAPP<sub>swe</sub> male rats. A–D: Hidden version of the Morris water maze for Fischer (□) and TgAPP<sub>swe</sub> (■) male rats. A: Acquisition, mean time ( $\pm$ SEM) to locate the escape platform ( $P = 0.046$ ). B: Percent time ( $\pm$ SEM) spent in each quadrant for Fischer rats during the probe trial. C: Percent time ( $\pm$ SEM) spent in each quadrant for TgAPP<sub>swe</sub> rats during the probe trial. D: Latency to cross training site ( $\pm$ SEM) for Fischer and TgAPP<sub>swe</sub> rats during the probe trial. E: Mean time ( $\pm$ SEM) to locate the escape platform during a visible version of the MWM ( $P = 0.589$ ). Quadrants are target (training) quadrant (Trgt), adjacent right, adjacent left, and opposite quadrant (Opp).  $P$  values for B and C derived from Tukey's pairwise multiple comparison test comparing percent search time in target quadrant with opposite; target quadrant with right; target quadrant with left following one-way ANOVA.

age. As shown in Figure 7A, control Fischer rats demonstrate impaired acquisition of the MWM task when compared with TgAPP<sub>swe</sub> rats ( $F_{(1,63)} = 6.023$ ,  $P < 0.04$ ). Furthermore, control Fischer rats show a disorganized search pattern in the probe trial and failed to show selectivity toward the target quadrant (see Figure 7B). In contrast, TgAPP<sub>swe</sub> rats show significant bias toward the target quadrant over all the remaining quadrants (see Fig-

ure 7C,  $P < 0.002$ , Tukey's pairwise multiple comparison test comparing percent search in target quadrant with opposite, target quadrant with right, and target quadrant with left following one-way ANOVA). Analysis of latency to cross the training site for the 1st time during the probe trial shows that the TgAPP<sub>swe</sub> rats found the location of the hidden platform significantly faster than control Fischer rats (see Figure 7D, Fischer mean latency =  $38.28 \pm 8.10$  s,



**Figure 4.** Social recognition in 6-mo-old Fischer (□) and TgAPP<sub>swe</sub> (■) male rats. Mean ( $\pm$ SEM) duration of investigation of the juvenile by 6-mo-old rats on the 1st exposure (0 min) and the 2nd exposure performed 5 min later (A) and 30 min later (B).



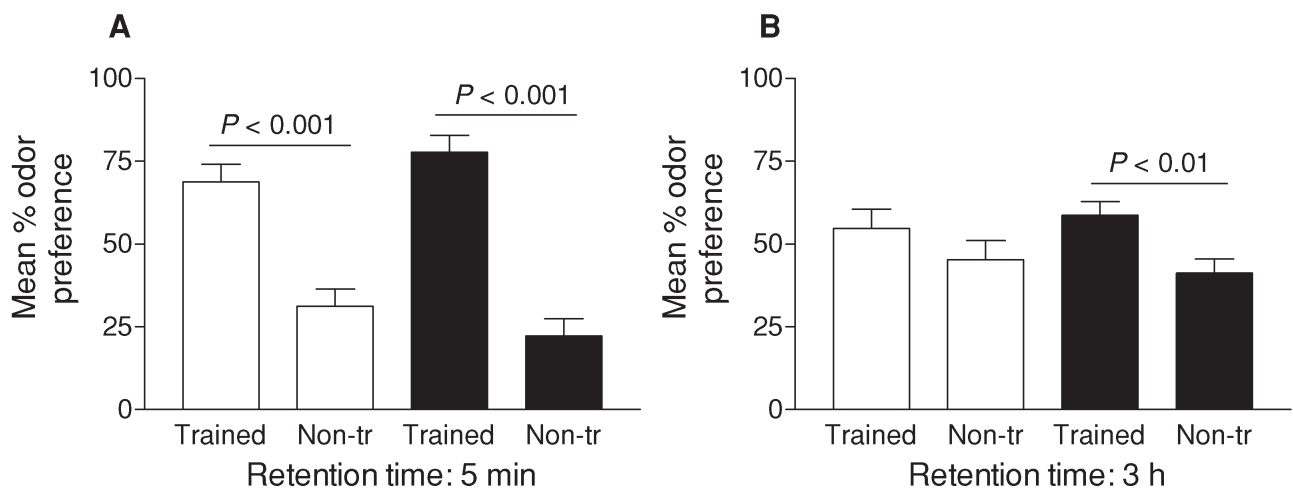
**Figure 5.** Social recognition in 12-mo-old Fischer (□) and TgAPPswE (■) male rats. Mean ( $\pm$ SEM) duration of investigation of the juvenile by 12-mo-old rats on the 1st exposure (0 min) and the 2nd exposure performed 5 min later (A) and 30 min later (B).

TgAPPswE mean latency =  $13.56 \pm 4.45$ ,  $F_{(1,18)} = 7.159$ ,  $P < 0.02$ ) corroborating better navigational performance in TgAPPswE compared with control Fischer rats. The observed differences are likely to be true differences in spatial learning and memory because both groups exhibited similar proficiency in locating the escape platform in the visible platform version of the MWM task (Figure 7E,  $F_{(1,99)} = 0.065$ ,  $P = 0.805$ ) and no significant difference in swim speeds were detected (swim speeds in probe trial: Fischer =  $19.56 \pm 1.07$  cm/s; TgAPPswE =  $21.93 \pm 1.02$  cm/s,  $F_{(1,18)} = 2.554$ ,  $P = 0.127$ ).

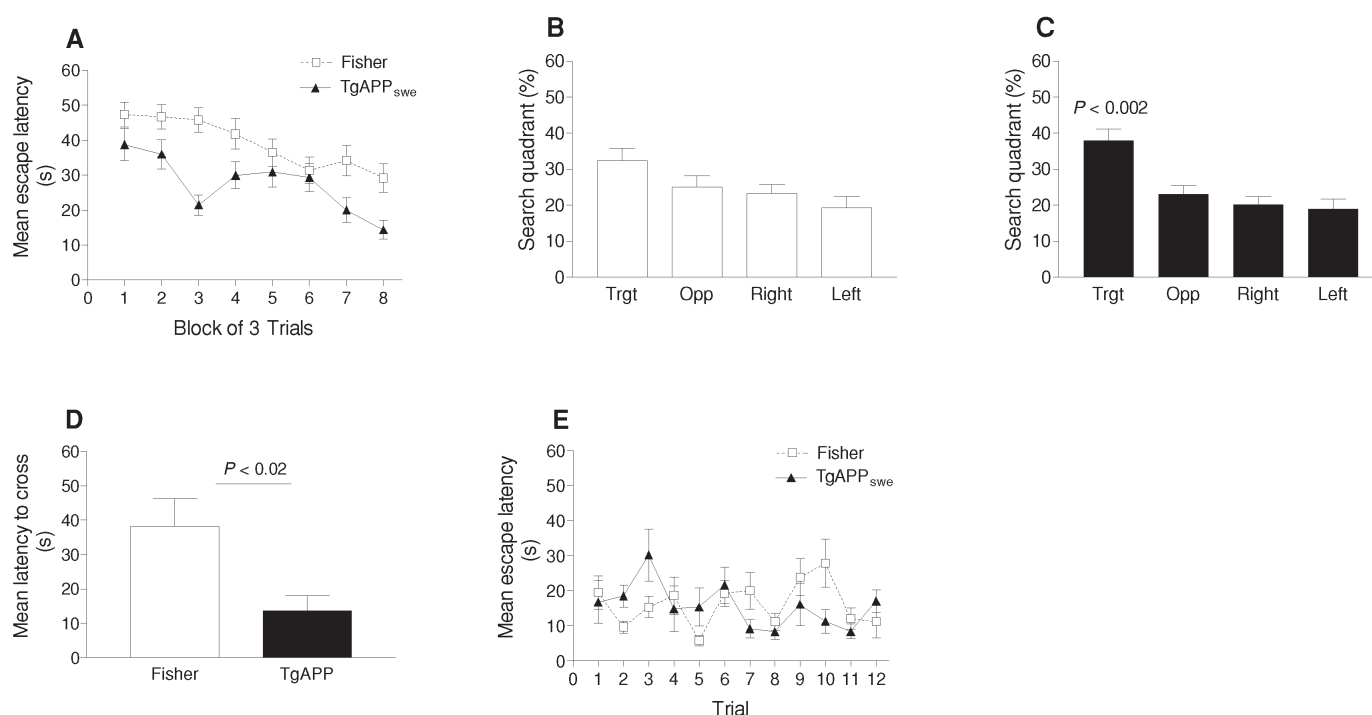
## DISCUSSION

Our study shows that transgenic human APPswE inbred Fischer-344 male rats with mild increases in APP and A $\beta$  levels but no extracellular amyloid deposits exhibit attenuated age-dependent cognitive decline when compared with age-matched control Fischer-344 male rats. Using 2 independent study groups, better cog-

nitive performance in 6-mo-old TgAPPswE rats was corroborated at 12 mo. In fact the difference in cognitive performance became more evident at 12 mo because control Fischer-344 rats declined further between 6 and 12 mo whereas no significant change in performance was detected between 6- and 12-mo-old TgAPPswE rats. Currently, we cannot rule out an age-dependent transgene effect. The learning and memory performance difference appeared limited to hippocampus-dependent behavioral tasks (STFP and MWM), and no difference was observed in a hippocampus-independent memory task (SR). These data contrast observations in 2 APPswE transgenic mouse models (7,9,10), which exhibited significant decline of cognitive performance in the MWM task at 3 mo (TgAPP23) and 9 to 10 mo of age (Tg2576)—a decline that could be attributed to the much higher levels ( $\geq 5$ -fold increase) of APPswE expression, which results in marked increases in A $\beta$  levels, extracellular amyloid deposits, and senile plaques in both mouse models (7,9,10).



**Figure 6.** Social transmission of food preference in 12-mo-old Fischer and TgAPPswE rats. Mean percent odor preference ( $\pm$ SEM) in 12-mo-old Fischer (□) and TgAPPswE (■) observers at 5 min (A) and 3 h (B) retention times. Non-tr, non-trained odor.



**Figure 7.** Spatial learning in 12-month-old Fischer and TgAPP<sub>swe</sub> male rats. A–D: Hidden version of the Morris water maze for Fischer (□) and TgAPP<sub>swe</sub> (■) male rats. A: Acquisition, mean time (±SEM) to locate the escape platform for Fischer rats during the probe trial. C: Percent time (±SEM) spent in each quadrant for TgAPP<sub>swe</sub> rats during the probe trial. *P* values for B and C derived from Tukey's pairwise multiple comparison test comparing percent search time in target quadrant with opposite; target quadrant with right; target quadrant with left following one-way ANOVA. D: Latency to cross training site (±SEM) for Fischer and TgAPP<sub>swe</sub> rats during the probe trial. E: Mean time (±SEM) to locate the escape platform during a visible version of the MWM (*P* = 0.805). Quadrants are targeted or training quadrant (Trgt), adjacent right, adjacent left, and opposite quadrant (Opp).

To date, neither the normal function(s) of APP, nor the mechanism(s) underlying cognitive impairment in AD is fully understood. The extracellular deposition of A $\beta$  correlates poorly with memory deficits in AD patients (29–31), and soluble A $\beta$  may correlate better with neural dysfunction and memory impairment (32–35). In one transgenic APP<sub>swe</sub> mouse model, cognitive impairment preceded increased A $\beta$  levels and plaque formation (10). One hypothesis regarding the role of A $\beta$  in AD is that oligomeric forms of A $\beta$  impair long-term potentiation and hence learning (36). Another study reports that excessive A $\beta$  results in synaptic depression contributing to cognitive decline in AD (3). We hypothesize that through these putative functional relationships—with long-term potentiation and/or excitatory synaptic transmission—it is possible that slight elevations of APP and/or A $\beta$  levels, that do not lead to oligomerization or extracellular amyloid deposits and plaque formation, may positively modulate or contribute to cognitive performance.

Alternatively, mechanisms related to APP may also explain the data. Increased expression of APP<sub>swe</sub> or any of its other metabolites may contribute to the detected behavioral phenotype as detected in experimental model studies. Improvement in MWM performance has been detected in 6- to 12-month-old transgenic mice with 5-fold induction of wild-type APP compared with age-matched nontransgenic mice (37). Analysis of the decline in cognitive function in Fischer-344 rats detects an association with

decreased cerebrospinal  $\alpha$ -secretase-cleaved soluble APP ( $\alpha$ -sAPP) levels, while A $\beta$  levels remained unchanged (38), thus implying a role for soluble APP in hippocampus-dependent cognition. These observations are concordant with the hypothesis that increased APP levels could underlie the improvement in hippocampus-dependent performance in TgAPP<sub>swe</sub> rats. Cumulative evidence pointing to the neurotrophic role of APP (39) delineates a putative mechanism for said hypothesis. Support for a neurotrophic role even for mutant APP<sub>swe</sub> is detected in 8-month-old TgAPP23 mice wherein high-level (5-fold increase) transgene expression of APP<sub>swe</sub> results in 13% more neocortical neurons compared with age-matched nontransgenic mice prior to the development of amyloid plaques (40). Further studies of TgAPP<sub>swe</sub> rats at later time points are necessary to elucidate the full spectrum effects of low-level expression of APP<sub>swe</sub> on cognitive performance as well as on APP metabolism.

Altogether, the data suggest the hypothesis that APP or its metabolites, including soluble A $\beta$  peptides, might have a physiological role in learning and memory function within a “narrow window” of expression; and that departure from this set level beyond a putative “threshold” could eventually lead to neuronal dysfunction with concomitant decline in cognitive function. The fact that A $\beta$  peptides are detected in cerebrospinal fluid and plasma in normal individuals throughout life suggesting a physiological role (2), and the fact that increased APP improves cogni-



tive function in TgAPP (wild-type) mice (37) support the emerging hypothesis for physiological role(s) of APP and its metabolites in cognition, mandating further study.

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