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Research report

Age-progressing cognitive impairments and neuropathology in transgenic CRND8 mice

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Abstract

Patients with Alzheimer's disease suffer from progressive cognitive impairments and show distinct post-mortem neuropathology, including β-amyloid plaques. Transgenic (Tg) CRND8 mice carry a mutated human amyloid precursor protein gene and show age-related increases in β-amyloid production and plaque deposition. It was previously reported that during the early stages of plaque deposition, Tg CRND8 mice demonstrated Morris maze impairments. However, it is unknown if Tg mice would be impaired at an earlier age prior to plaque deposition or more impaired at a later age with more extensive plaque deposition. In the current study, we describe Tg CRND8 age-progressing β-amyloid neuropathology and cognitive abilities in greater detail.

At all ages, Tg mice showed normal short-term memory in the Y-maze. Pre-plaque Tg and age-matched Non-Tg mice did not differ in learning the spatial Morris water maze. However, both early and late plaque Tg mice showed impairments during acquisition. In addition, although early plaque Tg mice performed well in the probe trial, late plaque Tg mice demonstrated impaired probe trial performance. Therefore compared to their Non-Tg littermates, Tg CRND8 mice demonstrate cognitive impairments that progressed with age and seemed to coincide with the onset of β-amyloid plaque deposition.

Keywords: Amyloid burden; Locomotor activity; Open field; Rota-rod; Reference memory; Novelty; Recognition; Sandwich immunoassay; Immunohistochemistry

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1. Introduction

Alzheimer's disease (AD) is the most common form of dementia among people over the age of 65 [12], with AD patients suffering from progressive loss of memory function and cognitive abilities [34]. AD is confirmed post-mortem by the presence of distinct neuropathological hallmarks, including senile plaques, neurofibrillary tangles, and neuronal cell loss, all of which progress with age. The exact causes of AD are unknown [3,33], but accumulating evidence suggests the involvement of β-amyloid (Aβ) peptides. Aβ peptides are produced by proteolytic cleavage of the amyloid precursor protein (APP) and $A\beta_{42}$ is the major constituent of senile plaques [27,33]. Furthermore, familial Alzheimer's disease (FAD) mutations of the APP, presenilin (PS) 1 and PS2 genes increase $A\beta_{42}$ production and lead to early-onset familial AD [31].

Several FAD mutations in human APP, PS1 and PS2 genes have been successfully over-expressed in mice, either alone or in combination (e.g., Tg2576: [17], PDAPP: [13]). APP FAD transgenic mouse models (with APP expression alone or together with PS1 or PS2 FAD mutations) show progressive, age-related increases in Aβ production, amyloid plaque pathology, astrogliosis, microgliosis and dystrophic neurites that are similar to what has been observed in AD patients. However, it is important to note that these mouse models of AD do not show certain AD-related pathological hallmarks, such as neurofibrillary tangles or substantial neuronal loss; hence, these mice model only certain aspects of the neuropathology that characterizes AD in humans [4,14,15].

As a cardinal feature of AD in humans, genetic mouse models of AD-like neuropathology should also show a progressive, age-related impairment in cognitive function [2,20]. Indeed, many studies have reported a progressive, age-related decline in certain aspects of cognitive function in transgenic mice expressing FAD mutations in the human APP gene with or without FAD mutated PS1 or PS2 genes [2,14,20]. The presence of progressive age-related cognitive impairments that parallel the progressive age-related neuropathology present in these models suggests that some aspect(s) of the amyloid neuropathology may be causing the decline in cognitive abilities.

Among studies which demonstrated progressive age-related impairments in certain cognitive measures in various transgenic AD mouse models displaying amyloid neuropathology, several reported that the impairments developed after an age at which plaque deposition had begun (e.g., PS1APP [1,29]; Tg2576 [5,8,10,28]; PDAPP [6,11]; APP23 [23]; PS2APP [30]), while others have demonstrated that the impairments preceded the onset of amyloid plaque deposition (e.g., Tg2576 [17,25,36]; PDAPP [18]; APP23 [35]; PS1Tg2576 [10]). Additionally, there have been reports that cognitive impairments were independent of age, either present at all ages tested regardless of pathology (e.g., TgCRND8 [19]), or not present at any of the ages tested (e.g., Tg2576 [24]; APPswe [32]). Overall, the relationship between amyloid neuropathology and cognition in transgenic AD mouse models is complex and requires further study.

One mouse model of AD-like amyloid neuropathology is the transgenic (Tg) CRND8 mouse. These mice over-express an APP gene containing the Swedish (K670N and M671L) and the Indiana (V717F) FAD mutations and show an age-related increase in Aβ production, as well as an early onset of plaque deposition in the cortex and hippocampus [7]. Up to 8 weeks of age, Tg CRND8 mice have elevated levels of $A\beta_{40}$ and $A\beta_{42}$, but no plaque deposition. From approximately 9 weeks of age, plaque deposition begins and progresses in these mice such that by 16 weeks, all Tg mice show multiple plaque deposits. Although Chishti et al. [7] provided a description of plaques in Tg mice as old as 32 weeks, quantification of the progression of Aβ plaque burden across a wider range of ages was not presented. In addition, it is not known how levels of cortical Aβ change after 26 weeks of age or how plasma Aβ levels change with age in Tg CRND8 mice.

Tg CRND8 mice have also demonstrated cognitive impairments [19] and abnormalities in synaptic plasticity [21] that seemed to be independent of age. At several ages after plaque deposition had begun (11–23 weeks), Tg CRND8 mice displayed impairments in the spatial version of the Morris water maze [7,19]. Since these were the only ages examined, it is not known if Tg CRND8 mice would show similar impairments at an earlier age, prior to plaque deposition. Further, it is also unknown if the Tg CRND8 Morris maze impairments are progressive in nature, such that at a later age when plaque deposition is more extensive in these mice, the cognitive impairments would be more severe than those seen in pre- or early plaque mice. Moreover, the Janus et al. [19] study was a longitudinal study (i.e., the mice were repeatedly tested in the same task at each age), allowing for the possibility that performance at the later ages may have been influenced by previous testing experiences in the maze.

The current study sought to replicate and, more importantly, extend the neuropathological and behavioral abnormalities observed in Tg CRND8 mice. By examining a wider range of ages (pre-plaque through late plaque; 6–50 weeks) and using a cross-sectional design (i.e., different mice were tested at different ages), we set out to characterize the progression of Aβ-related neuropathology and to determine if the spatial Morris maze impairments previously reported in early plaque Tg CRND8 mice were present prior to plaque deposition and progressed with age. In addition, locomotor activity, accelerating rota-rod performance, and short-term memory in the Y-maze were assessed in these mice throughout the age range.

2. Methods

2.1. Subjects

Tg and Non-Tg CRND8 mice were bred at the Schering-Plough Research Institute in Milan, Italy (for immunohistochemistry) or

Kenilworth, NJ (for sandwich immnoassay and behavior) from breeders originally obtained from Dr. David Westaway at the Centre for Research in Neurodegenerative Diseases at the University of Toronto. Mice were maintained on a mixed C57BL/6 and C3H/He background, such that offspring were produced by mating Tg males with B6C3H F1 females (Charles River Laboratories). For the sandwich immunoassay and behavioral studies, mice were weaned at 3–4 weeks, singly housed with a plastic igloo at 5–7 weeks until sacrifice or testing in a humidity (50%) and temperature (22 °C) controlled vivarium with a 12 h light/ dark cycle (lights on at 0700 h). Food, placed on the cage floor, and water were available ad libitum. For the behavioral studies, all mice were male, either heterozygous or wild type for the retinal degeneration gene carried by the C3H/He background strain and were littermates whenever possible and testing took place between 0800 and 1600 h. All in vivo procedures adhered to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Schering-Plough Research Institute, an AAALAC accredited institution.

2.2. β-Amyloid burden: immunohistochemistry

2.2.1. Tissue preparation

Four age groups of Tg mice were used: 9, 15, 25, and 40–50 weeks. Mice were anesthetized with 400 mg/kg tribromoethanol (Sigma) via the intraperitoneal route and transcardially perfused first with saline and then with 4% paraformaldehyde. After removal, brains were post-fixed in the same fixative (4 h), then washed with 0.01 M PBS, 50% ethanol and 70% ethanol and finally stored in 70% ethanol at 4 °C until embedding. Brains were embedded in paraffin (VIP, Miles Scientific) and 8 μm serial coronal sections were collected from the anterior pole to the cerebellum with a microtome.

2.2.2. Immunohistochemistry

Sections were incubated overnight at 4 °C with mouse anti-Aβ monoclonal antibody (Mab1561, recognizing residues 17–24 of Aβ, Chemicon, 1:100) to identify Aβ plaques. After washing in PBS, slides were incubated with biotinylated horse anti-mouse antibody (BA-2000, Vector Laboratories, 1:200) followed by ABC reagent (Vector Laboratories) for 30 min at room temperature. Sections were developed using DAB peroxidase (Sigma).

2.2.3. Image analysis

Time progression of Aβ burden in the Tg CRND8 mouse brain was evaluated at each of the four ages. At similar locations in the brain, three slices per mouse, separated by about 320 μm were quantified and averaged. Immunostained sections were viewed through a CCD video camera connected to a microscope (10× objective) and images were processed with a computerized image analysis system (Image Pro Plus Software, Media Cybernetics). For Aβ burden quantification, cortex (left and right hemisphere separately) was reconstructed for each slice using Corel Photo-Paint 9 software. Total cortical area and individual plaques were manually outlined up to the rhinal fissure, and the percentage of cortical area occupied by plaques was automatically calculated.

2.2.4. Data analysis

Data were analyzed with one between-subjects (age) analyses of variance (ANOVA). Following a significant omnibus *F*-test, a post hoc Fisher test was used to further analyze specific age differences.

2.3. β-Amyloid: sandwich immunoassay

2.3.1. Sandwich immunoassay

Three age groups of mice were used: 7–8, 20–24 and 43–44 weeks. The 43–44 week-old mice had extensive behavioral testing prior to sacrifice, while the 7–8 and 20–24 week-old mice were experimentally naïve. The mice were sacrificed by excess CO_2 and blood from the vena cava was immediately collected into heparinized tubes and kept on ice until centrifugation. Following centrifugation, plasma was extracted. The brain was dissected and the cortex was surgically isolated. Each cortex was homogenized in sucrose, extracted with formic acid and sonicated. Protein concentration was determined by the BCA assay (Pierce) [37].

2.3.2. Data analysis

Plasma Aβ data were analyzed with one between-subjects (age) ANOVA and following significant omnibus *F*-tests, post-hoc Fisher tests were used to further analyze specific age differences. Cortical Aβ data for 20–24 and 43–44 week-old mice were analyzed with a *t*-test since peptide levels were undetectable in 7–8 week-old Tg mice.

2.4. Locomotor activity and accelerating rota-rod

2.4.1. Subjects

Three age groups of male Tg and Non-Tg CRND8 mice were behaviorally tested together: pre-plaque (8 weeks), early plaque (21–22 weeks) and late plaque (38–42 weeks). For this and the other behavioral assays discussed below, we chose the name for each age group (e.g., "early plaque") based on the particular stage of plaque development in which the Tg mice of that age group were. Non-Tg mice are also part of each age group discussed, but Non-Tg mice do not display Aβ-related neuropathology at any of these ages (data not shown). All mice had been previously tested in the Morris water maze. The mice were first tested in the locomotor activity assay, followed by the accelerating rota-rod assay the next day.

2.4.2. Locomotor activity

Mice were individually placed in a small arena (26 cm × 26 cm × 39 cm; Coulbourn Instruments, Allentown, PA) and allowed to explore for 1 h during the light phase. The computer automatically calculated total distance traveled in 5 min bins.

2.4.3. Accelerating rota-rod

Mice were placed on a rod (d = 3 cm, l = 11 cm) that gradually and steadily increased in speed from 0 to 40 revolutions per min (rpm) over 5 min (AccuScan Instruments, Columbus, OH). The mice were required to ambulate on the rotating rod to avoid falling onto a wire grid 32 cm below. If the mice passively rotated (grabbed the bar and rotated without having to ambulate), they were gently pushed off, thus ending the trial. They were given eight consecutive trials with a 1 min inter-trial interval (ITI). The rpm at the time the mouse fell from the rod (or was pushed, in the case of passive rotation) was recorded for each trial.

2.4.4. Data analysis

Data were analyzed with two between-subjects (age and genotype) and one within-subjects (5 min blocks or trials) repeated measures ANOVA. Following significant omnibus *F*-tests, post-hoc

Fisher tests were used to further analyze specific age differences or age × genotype interactions.

2.5. Spatial Y-maze

2.5.1. Subjects

Two age groups of male Tg and Non-Tg CRND8 mice were behaviorally tested in separate studies: early plaque (16–22 weeks) and late plaque (42–46 weeks). Preliminary data suggested that both Tg and Non-Tg mice can be tested twice in the Y-maze with a 3-day separation in between sessions before the task loses its novelty (data not shown). Therefore, two cohorts of early plaque mice (60 and 90 min ITI; 75 min ITI) and two cohorts of late plaque mice (60 and 75 min ITI; 75 and 90 min ITI) were tested. All cohorts of early plaque mice were experimentally naïve, while the late plaque mice had been previously tested in several behavioral tasks (e.g., Morris maze, locomotor activity, accelerating rota-rod, object recognition memory (data not shown)) prior to testing in the Y-maze.

2.5.2. Spatial Y-maze

The testing procedure was based on Dellu et al. [9]. The maze (Med Associates Inc., Georgia, VT) was shaped like a "Y", with three equally spaced arms (35 cm × 7 cm × 13 cm; $l \times w \times$ h) radiating from a hexagonal center section ($d = 16$ cm). The arms were white with clear walls; the center section also had a white floor, but had metal opaque walls. The maze was located in a dimly lit (∼7 lux) room with extra-maze cues. Each mouse was given two testing sessions, separated by 3 days. For each testing session, subjects were given two trials. To begin a trial, the mouse was placed in a start box (17 cm × 7 cm × 13 cm) at the distal end of an arm (start arm; determined semi-randomly). After 10 s, a door was raised, allowing the mouse to enter the maze. After the mouse left the start box, the door was lowered and the 5 min trial began. During the first trial, the mouse was allowed to freely explore two of the three arms (determined semi-randomly). After a specific ITI (ranging from 60–90 min), the mouse was given a second 5 min trial, where all three arms were now available for the mouse to freely explore. Percent of total time (not including center time) spent in each arm for each trial was recorded. Since mice prefer novelty, if the mouse recognized and remembered which arm was novel during trial 2, the mouse should spend more time in the novel arm than would be expected by chance.

2.5.3. Data analysis

The percent of total arm time spent in the novel arm during trial 2 was compared to chance (33.3%) for Tg and Non-Tg mice with one-way *t*-tests. If significant, the group was considered to have recognized novelty and remembered this information for the duration of the ITI.

2.6. Morris maze

2.6.1. Subjects

In separate studies, three age groups of experimentally naïve male Tg and Non-Tg CRND8 mice were behaviorally tested: pre-plaque (6–8 weeks), early plaque (19–22 weeks) and late plaque (39–40 weeks). Two cohorts of 6–8 week-old mice, two cohorts of 19–22 week-old mice, and one cohort of 39–40 week-old mice were tested. There were no significant learning differences between the two 6–8 week-old and the two 19–22 week-old cohorts, so data were combined into one 6–8 and one 19–22 week-old group.

2.6.2. Morris maze

The testing procedure was based on that of Janus et al. [19]. A custom-made pool ($d = 112$ cm) with seamless, transparent walls was located in a well lit room with numerous extra-maze cues (national flags). The water (20–22 °C), which was made opaque with non-toxic white paint, was 8.5 cm from the top of the pool edge. The white plastic escape platform ($d = 9.5$ cm), covered with fine plastic mesh painted white, was located 1 cm below the water surface in the center (21.6 cm from the edge of the platform to the pool wall) of one of four pool quadrants (NE, SE, SW, and NW). For each trial, a mouse was released from any of four semi-randomly determined locations (N, S, E, and W) and had 60 s to locate the escape platform, where it was allowed to remain for 10 s. The mouse was then returned to its holding cage, which was located under an incandescent black heat lamp. If the platform was not found in the allotted time, the mouse was gently guided to it with a small plastic rod.

Experimentally naïve mice were first tested in the cued version of the maze in which mice had to locate a hidden escape platform that was marked with a three-dimensional black flag ($d = 27$ cm; $h = 17$ cm). The platform was moved semi-randomly to another quadrant after each trial. The mice were given four trials per day for 3 days with an ITI of 30–35 min. After 2 days without testing, the mice were subsequently tested in the spatial version of the maze in which mice were to locate a hidden escape platform that remained in a fixed position in the pool (target quadrant). The platform was located in the center of one of the four quadrants for different mice. The mice were given four trials per day for 5 days with an ITI of 30 min. Thirty minutes after the last trial of spatial testing on day 5, the platform was removed from the pool and each mouse was given a 60 s probe trial.

The mice were tracked in the pool by a HVS 2020 Plus video tracking system with Water 2020 software (HVS Image, Buckingham, UK). For the spatial version, measures of latency, path length, swimming speed, and time spent in the outer one-third annuli of the pool (measure of thigmotaxic behavior) were obtained. During spatial testing, each mouse was carefully observed for "floating" behavior (absence of active swimming behavior accompanied by a distinct posture, including extended limbs and/or tail) and those mice that displayed this behavior on at least one trial within a day were categorized as "floaters", with the remaining mice being categorized as "non-floaters". It is important to note that none of the "floaters" floated for more than a few seconds of the 60 s trial, therefore, data from all mice regardless of floating status were included in the data analyses. For the probe trial, percent time spent and percent of total platform crossings (two times the diameter of the platform) in each quadrant were obtained. Tg and Non-Tg mice were categorized as either "unimpaired" or "impaired" as determined by probe trial performance. Mice that had greater than 40% of platform crossings in the target quadrant during the probe trial were categorized as "unimpaired" [26]. The remaining mice were categorized as "impaired". This level of preferential searching for the platform in the correct area of the pool suggested that these mice used spatial cues while learning the spatial version of the maze. Mice using alternative strategies, such as an egocentric (swimming a certain distance from the pool wall) or random strategy, would not prefer the target area of the pool. Due to tracking problems in one cohort, only latency data are presented for cued learning.

2.6.3. Data analysis

Morris maze learning data (average of four daily trials) from each age group were analyzed with one between-subjects (genotype) and one within-subjects (days) repeated measures ANOVA. Linear trend analyses were used to determine if "learning" (significant linear days effect) had occurred and to further examine interactions with genotype. For the probe trial, within each genotype, percent time spent and percent total platform crossings in the target quadrant were compared to the next preferred quadrant with planned comparison *t*-tests. Within each age, the proportion of "unimpaired" and "impaired" mice and the proportion of "floater" and "non-floater" mice for the Tg group were compared to those proportions for the Non-Tg group with Fisher exact tests.

Since the different age groups of Tg and Non-Tg mice were tested at different times, we were not comfortable statistically testing for the effects of age on Morris maze performance. Instead, at each age, performance was compared to age-matched Non-Tg mice and impairments in acquisition of the task or probe trial performance were considered to be either present or absent. If additional aspects of Morris maze performance were impaired as the mice aged, we interpreted this as "age-progressing" Morris maze impairments.

3. Results

3.1. β-Amyloid burden

Fig. 1 shows representative coronal sections from 9, 15, 25, and 40–50 week-old mice. There was little evidence of Aβ plaques in 9 week-old mice. By 15 weeks, a few plaques had been deposited in the cortex and hippocampus. Deposition continued to increase at 25 weeks and was quite extensive when the mice were 40–50 weeks old. As the Tg mice aged, there was a significant increase in the percent of cortical area occupied by plaques (main effect of age: $F(3,12) = 89.32$, $p < 0.0001$), such that there was a statistically significant increase in Aβ burden from 15 to 25 weeks and from 25 to 40+ weeks ($p < 0.0003$), but the increase from 9 to 15 weeks failed to reach significance (Fig. 2A).

3.2. β-Amyloid

Plasma levels of $A\beta_{40}$ and $A\beta_{42}$ remained relatively constant across the three ages tested (main effect of age: n.s.; Fig. 2B). Levels of cortical $A\beta_{40}$ and $A\beta_{42}$ extracted by formic-acid significantly increased 3–4-fold from 20–24 to 43–44 weeks ($t(15) = 14.36$ and 18.60, $p < 0.0001$, for $A\beta_{40}$ and $A\beta_{42}$, respectively; Fig. 2C). Following formic acid extraction, the amount of $A\beta_{40}$ and $A\beta_{42}$ in the cortex of 7–8 week-old mice was below the level of detection. However, in other studies using guanidine extraction, levels of $A\beta_{40}$ and $A\beta_{42}$ were between 1–2 and 0.5–0.75 pg Aβ/μg cortex protein in 6–7 week-old Tg CRND8 mice (unpublished observations), which is elevated compared to Non-Tg mice, but lower than that observed in 20–24 week-old mice.

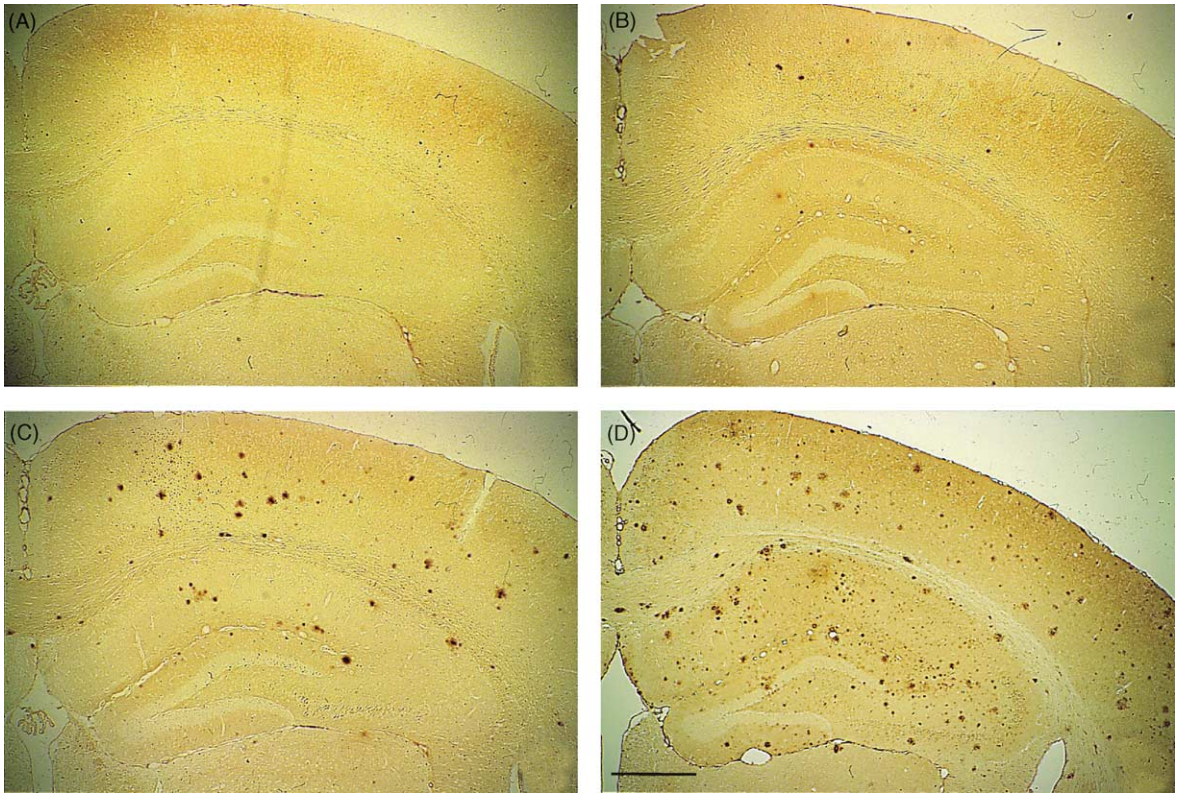

Fig. 1. Aβ plaque load and distribution (immunohistochemistry with Mab 1561) in the cerebral cortex and hippocampus of representative 9 (A), 15 (B), 25 (C), and 40 (D) week-old Tg mice. Scale bar = 500 μm.

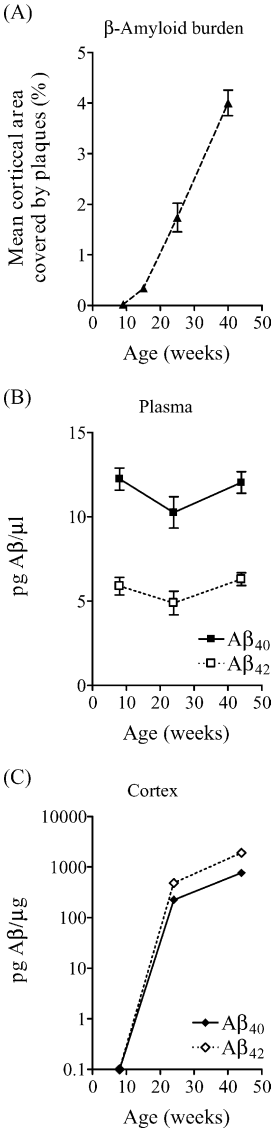

Fig. 2. (A) Cortical Aβ burden in 9 ($N = 4$), 15 ($N = 4$), 25 ($N = 4$), and 40–50 ($N = 4$) week-old Tg mice. (B and C) Plasma (B) and cortex (C) $A\beta_{40}$ and $A\beta_{42}$ levels in 7–8 ($N = 8$), 20–24 ($N = 7$), and 43–44 ($N = 10$) week-old Tg mice. Data are expressed as mean ± SEM. Note that although the levels of $A\beta_{40}$ and $A\beta_{42}$ in the cortex of 7–8 week-old mice are graphed as 0.1 pg/μg, they were actually not detectable following formic acid extraction.

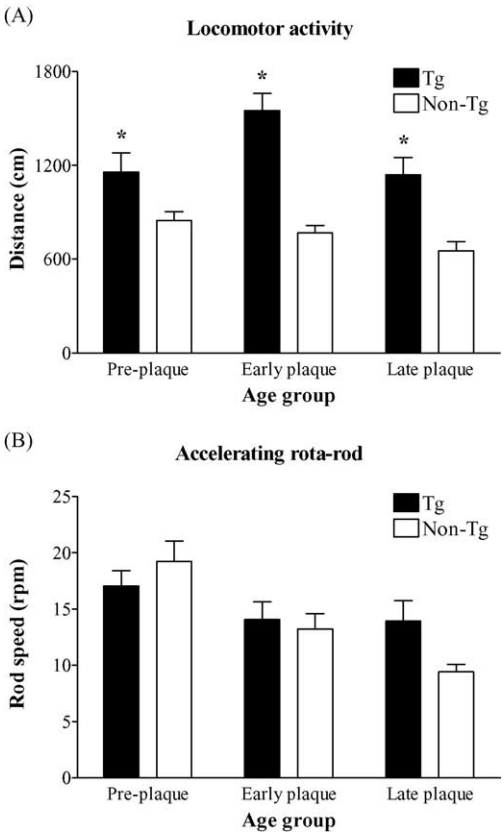

Fig. 3. (A) Locomotor activity and (B) accelerating rota-rod performance in pre-plaque (8 weeks; 9 Tg and 10 Non-Tg), early plaque (21–22 weeks; 10 Tg and 10 Non-Tg) and late plaque (38–42 weeks; 7 Tg and 5 Non-Tg) Tg mice (filled bars), as well as age-matched Non-Tg mice (open bars). Data are expressed as mean ± SEM. Significantly different from Non-Tg mice of the same age, $^{*}p < 0.05$.

3.3. Locomotor activity and accelerating rota-rod

Regardless of genotype, age affected how active the mice were (main effect of age: $F(2,45) = 3.93$, $p < 0.03$) with 21–22 week-old mice ($N = 20$) being more active than 38–42 week-old mice ($N = 12$) ($p < 0.03$) and slightly more active than 8 week-old mice ($N = 19$) ($p < 0.06$). Regardless of age, Tg mice ($N = 26$) were more active during the 1 h test than Non-Tg mice ($N = 25$) (main effect of genotype: $F(1,45) = 46.86$, $p < 0.0001$; genotype × block interaction: $F(11,495) = 2.16$, $p < 0.02$). Although the difference between Tg and Non-Tg mice was the largest in 21–22 week-old mice (age × genotype interaction: $F(2,45) = 3.95$, $p < 0.03$), Tg mice were more active than Non-Tg mice at each of the three ages ($p < 0.02$; Fig. 3A).

For the accelerating rota-rod, there were no significant differences between Tg and Non-Tg mice. However, as the mice aged (regardless of genotype), their rota-rod performance declined (main effect of age: $F(2,45) = 8.17$, $p < 0.0009$), with 8 week-old mice ($N = 19$) performing better than 21–22 week-old mice ($N = 20$), which in turn performed better than 38–42 week-old mice ($N = 20$) ($p < 0.004$; Fig. 3B).

3.4. Spatial Y-maze

3.4.1. Early plaque mice (16–22 weeks)

Both Tg ($N = 7$) and Non-Tg ($N = 7$) 16–22 week-old mice spent more time in the novel arm than expected by chance after the 60 min ITI ($t(6) = 3.02$ and 2.42, $p < 0.05$), but not after 90 min ($t(6) = -0.73$ and 0.82, n.s.). After 75 min, Tg ($N = 5$) ($t(4) = 2.56$, $p < 0.05$), but not Non-Tg ($N = 6$) ($t(5) = 1.15$, n.s.), mice spent more time in the novel arm than expected by chance (Fig. 4A).

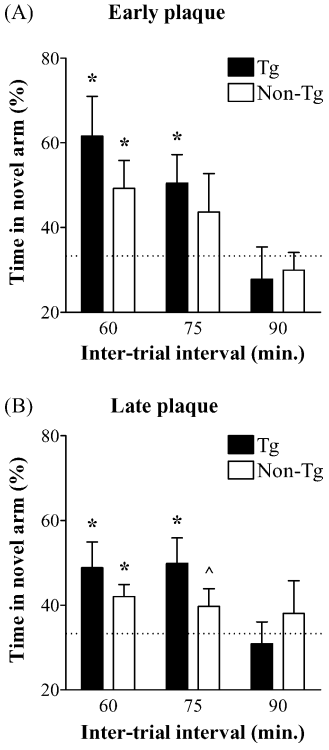

Fig. 4. Spatial Y-maze short-term memory in (A) early plaque (16–22 weeks) and (B) late plaque (42–46 weeks) Tg (filled bars) and Non-Tg (open bars) mice. Early plaque: 7 Tg and 7 Non-Tg for 60 and 90 min ITI; 5 Tg and 6 Non-Tg for 75 min ITI. Late plaque: 10 Tg and 10 Non-Tg at 60 and 90 min ITI and 20 Tg and 20 Non-Tg at 75 min. Dotted line = chance (33.3%). Data are expressed as mean ± SEM. Different from chance, $^{*}p < 0.05$; $\hat{}p < 0.10$.

3.4.2. Late plaque mice (42–46 weeks)

Similar to what was found with the early plaque mice after a 60 min ITI, both Tg ($N = 10$) and Non-Tg ($N = 10$) 42–46 week-old mice spent more time in the novel arm than expected by chance ($t(9) = 2.55$ and 3.09, $p < 0.05$), but after a 90 min ITI, neither group preferred the novel arm ($t(9) = 0.47$ and 0.60, n.s.). The 75 min ITI was given first for one cohort and second for the other. Since a two-between subjects (genotype and session) ANOVA on the 75 min data failed to reveal any differences in percent time spent in the novel arm, the 75 min ITI data for two cohorts were combined. After the 75 min ITI, Tg ($N = 20$) ($t(19) = 2.74$, $p < 0.05$), but not Non-Tg ($N = 20$) ($t(19) = 1.53$, $p < 0.10$), mice spent significantly more time in the novel arm than expected by chance (Fig. 4B).

3.5. Morris maze

3.5.1. Pre-plaque mice (6–8 weeks)

In the cued version of the Morris maze, there were no learning differences between 6–8 week-old Tg ($N = 17$) and Non-Tg ($N = 20$) mice (Fig. 5A), with both groups decreasing their latency scores over time (linear trend of days: $F(1,70) = 16.68$ and 40.48, $p < 0.001$, for Tg and Non-Tg mice, respectively) and not differing in the rate of decline (genotype × linear days interaction: n.s.). Escape latency data for the cued version were unavailable for three Tg mice because of technical problems. One Tg mouse died in between cued and spatial Morris maze testing.

In the spatial version of the Morris maze, there were no significant differences between 6–8 week-old Tg ($N = 19$) and Non-Tg ($N = 20$) mice in escape latency, path length or time spent in the outer annuli of the pool (Fig. 5A and Table 1). Both Tg and Non-Tg mice showed similar significant reductions in escape latency and path length over time (genotype × linear days: n.s. for latency and path length; linear days for Tg mice: $F(1,148) = 14.86$ (latency) and 18.12 (path length), $p < 0.001$; for Non-Tg mice: $F(1,148) = 15.03$ (latency) and 19.73 (path length), $p < 0.001$). Tg mice tended to swim faster than Non-Tg mice (main effect of genotype: $F(1,37) = 3.78$, $p < 0.06$; Table 1), but only on certain days (genotype × days interaction: $F(4,148) = 2.49$, $p < 0.05$; data not shown). These minor differences in swimming speed may be related to the fact that somewhat fewer Tg mice (11%) were categorized as "floaters" compared to Non-Tg mice (50%); this difference was marginally significant ($p < 0.07$).

During the probe trial, 6–8 week-old Tg ($N = 19$) ($t(18) = 3.98$, $p < 0.0009$), but not Non-Tg ($N = 20$), mice spent more time in the target quadrant than the next preferred quadrant (Table 1). Platform crossings occurred preferentially in the target quadrant for both Tg and Non-Tg mice ($t(18) = 3.52$, $p < 0.003$ and $t(19) = 2.46$, $p < 0.03$; Fig. 5A).

Of the 19 Tg and 20 Non-Tg 6–8 week-old mice tested in the probe trial, 11 (58%) Tg and 16 (80%) Non-Tg mice were considered to have learned the spatial version of the Morris maze using spatial cues (based on ≥40% of platform crossings made in the target quadrant during the probe trial). A Fisher's exact probability test revealed that this was not a significant difference, suggesting that the distribution of "unimpaired" and "impaired" mice did not differ between the genotypes at this age.

3.5.2. Early plaque mice (19–22 weeks)

In the cued version, there were no differences between 19–22 week-old Tg ($N = 20$) and Non-Tg ($N = 18$) mice in escape latency (Fig. 5B). Both Tg and Non-Tg mice improved their performance over test days and in a similar manner (linear days: $F(1,72) = 34.29$ and 23.59, $p < 0.001$, for Tg and Non-Tg mice, respectively; genotype × linear days interaction: n.s.). Latency data for the cued version were unavailable for two Non-Tg mice because of computer tracking problems. One Tg mouse died in between cued and spatial Morris maze testing.

In the spatial version, 19–22 week-old Tg mice ($N = 19$) took longer than Non-Tg mice ($N = 20$) to locate the hidden escape platform (main effect of genotype: $F(1,37) = 16.44$, $p < 0.0003$; Fig. 5B). Tg and Non-Tg mice showed different rates of decline in latency over time (genotype × linear days: $F(1,148) = 3.99$, $p < 0.05$) since Tg mice did not decrease their latency scores over days (linear days: n.s.) and Non-Tg mice did (linear days: $F(1,148) = 22.43$, $p < 0.001$). Tg mice

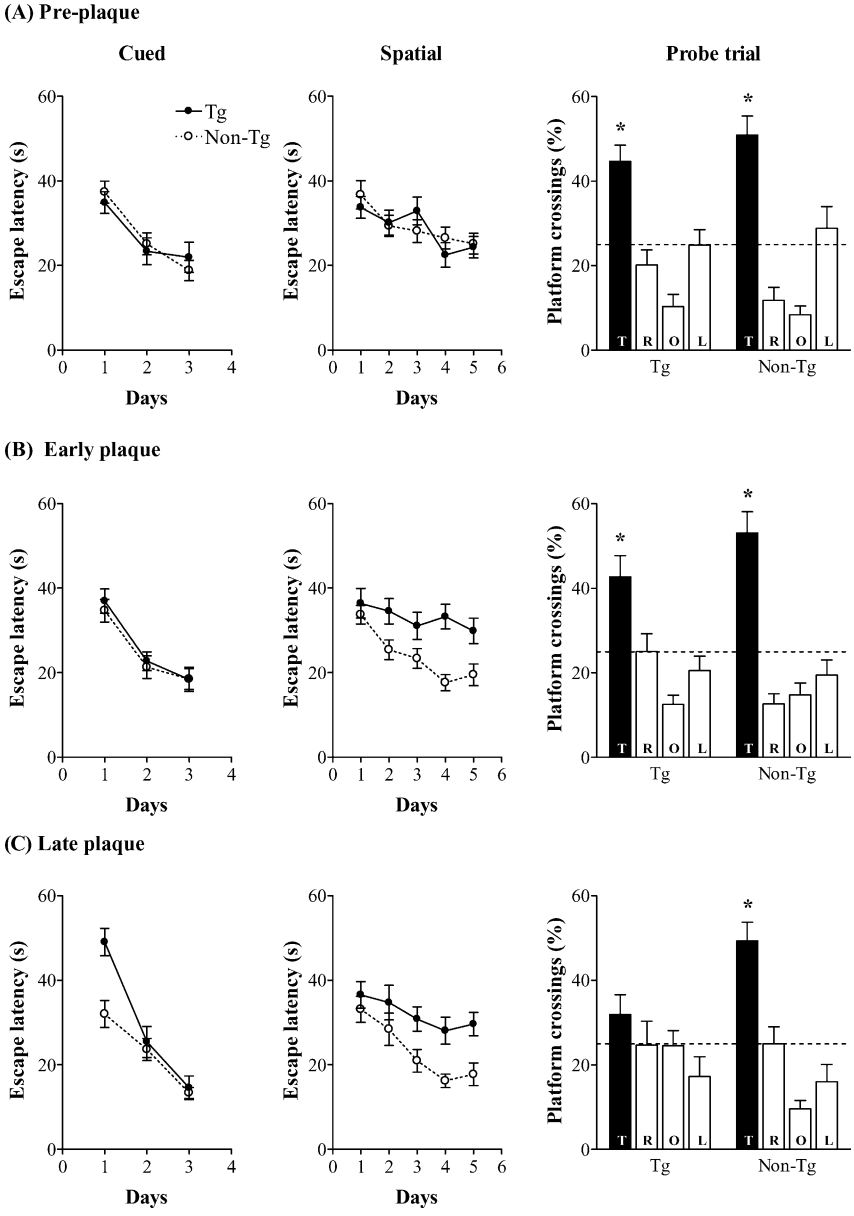

Fig. 5. Cued (left figures) and spatial (center figures) Morris water maze learning (escape latency) for Tg (●) and Non-Tg (○) CRND8 mice at (A) pre-plaque (6–8 weeks; 17 cued/19 spatial Tg and 20 Non-Tg), (B) early plaque (19–22 weeks; 20 cued/19 spatial Tg and 18 cued/20 spatial Non-Tg) and (C) late plaque (39–40 weeks; 14 cued/13 spatial Tg and 16 Non-Tg) ages. The right figures show percent platform crossings made in the target (T; solid black), right (R), opposite (O), and left (L) quadrants by Tg and Non-Tg mice during the probe trial, administered 30 min after spatial training concluded at the three different ages. Dotted line = chance (25%). Data are expressed as mean ± SEM. Significantly different from the next preferred quadrant, $^{*}p < 0.05$.

also had longer path lengths than Non-Tg mice (main effect of genotype: $F(1,37) = 15.01$, $p < 0.0005$; Table 1). However, both groups showed a similar and significant reduction in path length over days (genotype × linear days: n.s.; linear days: $F(1,148) = 11.36$ and 27.83, $p < 0.01$, for Tg and Non-Tg mice, respectively; data not shown). Tg and Non-Tg mice did not differ in swimming speed or in time spent in the outer annuli of the pool (Table 1). Eleven percent of Tg and 25% of Non-Tg mice were floaters; this is not a significant difference.

Despite 19–22 week-old Tg mice being impaired in learning the Morris maze compared to Non-Tg mice, Tg mice ($N = 19$) preferentially crossed the platform area of the target quadrant more than the next preferred quadrant ($t(18) = 2.23$, $p < 0.04$), but did not spend significantly more time in the target quadrant compared to the next preferred quadrant (Fig. 5B and Table 1). Non-Tg mice ($N = 20$) preferred the target quadrant more than the next preferred quadrant for both percent time and percent platform crossings ($t(19) = 2.84$ and 4.23, $p < 0.02$, for time and crossings, respectively; Fig. 5B and Table 1).

Eight of 19 Tg and 15 of 20 Non-Tg 19–22 week-old mice showed good use of spatial cues when learning the Morris

Table 1
Additional Morris water maze measures (mean ± SEM) for transgenic (Tg) and non-transgenic (Non-Tg) CRND8 mice at three ages

	6–8 Weeks		19–22 Weeks		39–40 Weeks	
	Tg	Non-Tg	Tg	Non-Tg	Tg	Non-Tg
Spatial version						
Path length (m)	6.37 ± 0.60	5.62 ± 0.38	6.54 ± 0.39	4.74 ± 0.26[a]	6.26 ± 0.49	4.33 ± 0.32[a]
Speed (m/s)	0.20 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
Time in outer annuli (%)	53.56 ± 2.32	57.97 ± 2.94	51.07 ± 2.11	49.44 ± 1.74	49.91 ± 2.17	49.12 ± 2.02
Probe trial						
Time (%)						
Target	44.88 ± 3.44[b]	38.06 ± 3.28	37.56 ± 3.92	40.39 ± 3.13[b]	30.53 ± 3.40	45.04 ± 3.48[b]
Right	21.86 ± 2.54	19.26 ± 3.01	26.21 ± 3.98	18.94 ± 2.57	26.22 ± 3.79	23.23 ± 2.97
Opposite	10.12 ± 2.08	14.51 ± 2.17	16.59 ± 2.60	14.73 ± 1.72	22.54 ± 3.02	11.23 ± 2.05
Left	23.07 ± 3.13	28.10 ± 3.93	19.56 ± 2.51	25.86 ± 2.87	20.58 ± 3.36	20.39 ± 3.99

[a] Significantly different from Tg mice, $p < 0.05$
[b] Significantly different from next preferred quadrant, $p < 0.05$.

maze as evidenced by performance during the probe trial. This distribution of "unimpaired" and "impaired" mice for the Tg group tended to differ from the Non-Tg group (Fisher's exact probability test, $p < 0.06$); only 42% of Tg mice were unimpaired, compared to 75% of Non-Tg mice.

3.5.3. Late plaque mice (39–40 weeks)

In the cued version, both Tg ($N = 14$) and Non-Tg ($N = 16$) 39–40 week-old mice reduced their latency scores over time (linear days: $F(1,56) = 96.17$ and 32.21, $p < 0.001$, for Tg and Non-Tg mice, respectively), but at different rates (genotype × linear days interaction: $F(1,56) = 10.79$, $p < 0.01$; Fig. 5C). Collapsed across days, Tg mice took longer than Non-Tg mice to locate the cued platform (main effect of genotype: $F(1,28) = 5.13$, $p < 0.04$; Fig. 5C). However, Tg mice swam longer only on day 1 ($t(28) = 3.72$, $p < 0.0009$), not on days 2 or 3 (genotype × days interaction: $F(2,56) = 6.94$, $p < 0.003$; Fig. 5C). One Tg mouse died in between cued and spatial testing.

In the spatial version, 39–40 week-old Tg mice ($N = 13$) exhibited longer escape latencies (Fig. 5C) and swam farther (Table 1) than Non-Tg mice ($N = 16$) (main effect of genotype: $F(1,27) = 10.68$ and 11.84, $p < 0.003$, for latency and path length, respectively). Both Tg and Non-Tg mice reduced their latency (linear days: $F(1,108) = 5.22$ and 28.34, $p < 0.05$, for Tg and Non-Tg mice, respectively) and distance ($F(1,108) = 7.07$ and 30.67, $p < 0.025$) scores over time, although at slightly different rates (genotype × linear days interaction: $F(1,108) = 3.49$ and 3.00, $p < 0.10$, for latency and path length, respectively). The genotypes did not significantly differ in swimming speed or in percent time spent in the outer annuli of the pool (Table 1). No Tg mice floated and only one of 16 Non-Tg mice floated (n.s.).

During the probe trial, 39–40 week-old Tg mice ($N = 13$) did not significantly prefer the target quadrant, while Non-Tg mice ($N = 16$) preferred the target quadrant more than the next preferred quadrant ($t(15) = 4.53$ and 3.41, $p < 0.004$, for percent time and platform crossings; Fig. 5C and Table 1).

Four of 13 Tg (31%) and 12 of 16 Non-Tg (75%) 39–40 week-old mice used spatial cues to learn the spatial version of the maze. This is a significant difference, with a lower percentage of Tg mice being categorized as "unimpaired" than Non-Tg mice (Fisher's exact probability test, $p < 0.03$).

4. Discussion

Chishti et al. [7] previously described age-related increases in plaque formation and Aβ production in the Tg CRND8 brain, thereby confirming this mouse as a model of Alzheimer's disease-like Aβ neuropathology. The current study extended these findings to quantify the Aβ burden in Tg mice just before the plaque deposition stage (9 weeks), in early plaque stages (15 and 25 weeks) and in a late plaque stage (40–50 weeks). It was shown that, as expected, there was a very significant age-related increase in cortical Aβ burden in Tg mice, such that by 40 weeks of age, the Aβ burden was quite extensive, covering ∼4% of cortical area as defined by immunohistochemical staining.

In our study, levels of plasma and cortical Aβ were assessed in pre-plaque (7–8 weeks), early mid-plaque (20–24 weeks) and late plaque (43–44 weeks) Tg CRND8 mice. It was revealed that the amount of $A\beta_{40}$ and $A\beta_{42}$ in the plasma remained unchanged during the age-related progression of cortical Aβ burden. This is in contrast to a report that plasma Aβ decreased with age in the Tg2576 mouse model of Alzheimer's disease [22]. The reasons for this discrepancy are unknown, but may be related to differences in the how old the mice were at the time of examination (i.e., the extent of plaque burden present). In addition, we found that levels of cortical $A\beta_{40}$ and $A\beta_{42}$ increased as the mice aged, which agrees with and extends the findings of Chishti et al. [7] using the Tg CRND8 mouse and is in agreement with what has been shown with other models as well (e.g., Tg2576 [22]). Moreover, the age-related increase in cortical Aβ peptides paralleled the age-related increase in Aβ burden.

In an effort to further behaviorally characterize the Tg CRND8 mouse, we assessed locomotor activity and accelerating rota-rod performance in pre-plaque (8 weeks), early plaque (21–22 weeks) and late plaque (38–42 weeks) mice.

We found that Tg mice were more active than Non-Tg mice at the three ages and the genotype difference did not increase with age, indicating that plaque deposition did not affect locomotor activity in Tg mice. Hyperactivity in other Tg mice expressing FAD mutations has been reported by other investigators (e.g., PS2APP [30]; PDAPP [11]). In a test of motor learning and coordination, the accelerating rota-rod, Tg mice did not differ from Non-Tg mice at the three ages, suggesting that Tg mice have normal motor skills prior to and throughout the plaque deposition period. Others have also found this to be the case (e.g., Tg2576 [10]).

The spatial Y-maze was used to assess short-term memory in Tg and Non-Tg mice at early (16–22 weeks) and late (42–46 weeks) post-plaque ages. Since mice prefer novel over familiar locations, mice should spend more time in the novel arm than would be expected by chance if they recognize and, most importantly, remember the novel arm after a given inter-trial interval [9]. On the other hand, if the mouse does not prefer the novel arm after a given inter-trial interval, one can conclude that either (1) the mouse does not recognize (or prefer) novelty or (2) memory for this information has decayed. To distinguish between these possibilities, it was important to demonstrate that each group of mice recognized and preferred novelty, regardless of the memory demand. To this end, we tested the mice with an inter-trial interval that was short enough (e.g., 1 h) for the mice to be able to remember the novel arm. We found that both early and late plaque Tg and Non-Tg mice were able to recognize and remember spatial novelty for at least 1 h, suggesting that both groups of mice preferred novelty and recognized the novel arm in our testing situation. However, when the memory demand increased such that the mice had to remember the novel arm for 90 min, memory for the information seemed to have decayed to a similar extent for both Tg and Non-Tg mice at both ages. Therefore, regardless of age and the degree of plaque deposition, Tg and Non-Tg mice showed very similar short-term spatial memory capacity up to 46 weeks of age. It should be noted, however, that the late plaque mice had previous testing experience in other tasks whereas the early plaque mice were experimentally naïve which may have masked potential genotype differences.

The Morris maze, as it was used here and previously [19], requires the use of "reference memory", which is needed to remember information that remains constant over time (i.e., the location of the hidden platform) [16]. Janus et al. [19] reported that early plaque Tg CRND8 mice took longer than Non-Tg mice to locate the escape platform in the spatial version of the Morris maze, but it was unknown if these impairments would be present in pre-plaque mice or would become more severe at later ages. The current study confirms and extends previous findings by examining Morris maze learning in Tg mice at an age when plaques had yet to be deposited (6–8 weeks), an age previously described by Janus et al. [19] when plaques have just begun to be deposited (19–22 weeks) and a much later age, when the Aβ plaque burden was high (39–40 weeks).

Pre-plaque (6–8 weeks) Tg and Non-Tg mice did not differ in learning the cued and spatial versions of the Morris water maze. When the platform was removed for the probe trial given after spatial testing concluded, both groups preferred the area of the pool where the platform was previously located indicating that most Tg and Non-Tg mice used spatial cues to learn the task. The proportion of mice that learned the task using spatial cues did not differ between the genotypes. Therefore, pre-plaque Tg mice were unimpaired in learning the Morris water maze.

Early plaque (19–22 weeks) Tg mice were impaired in learning the spatial version of the maze compared to Non-Tg mice, since Tg mice took longer and swam farther than Non-Tg mice to locate the hidden platform. Although Tg mice were impaired in learning the spatial version of the maze at this age, the probe trial showed that both Tg and Non-Tg mice preferred the area of the pool where the platform was previously located during spatial testing. Moreover, the proportion of Tg mice that used spatial cues to learn the maze was only marginally significantly different from that of Non-Tg mice. Thus, early plaque Tg mice were impaired in learning the spatial version of the maze, but the impairment did not extend to the probe trial test. These results with 19–22 week-old Tg CRND8 mice are very similar to those reported by Janus et al. [19] for 19 and 23 week-old Tg CRND8 mice.

Late plaque (39–40 weeks) Tg mice were also impaired in the spatial version of the Morris maze compared to Non-Tg mice at the same age, since Tg mice displayed longer escape latencies and longer path lengths than Non-Tg mice. Further, unlike Non-Tg mice, Tg mice did not prefer the target area of the pool during the probe trial, with a lower proportion of Tg mice compared to Non-Tg mice using spatial cues to learn the spatial version of the task. Taken together, late plaque Tg mice were not only impaired during acquisition of the spatial Morris maze task, but showed poor probe trial performance as well.

Overall, regarding performance in the spatial Morris maze compared to age-matched Non-Tg littermates tested at the same time, pre-plaque Tg mice were unimpaired during acquisition and the probe trial, early plaque Tg mice were impaired during acquisition, but were unimpaired during the probe trial, and late plaque Tg mice were impaired during both acquisition and the probe trial. Thus, as Tg mice aged, first they were impaired in one aspect of the task (acquisition) and later they were impaired in two aspects of the task (acquisition and probe trial). This suggested that Tg CRND8 mice show age-progressing deficits in the spatial Morris maze. Since each age group of mice was tested separately at different times of the year, we discourage direct comparisons across age within either the Tg or Non-Tg groups.

Since Tg mice did not significantly differ from Non-Tg mice in learning the cued version of the maze at the pre- and early plaque ages and late plaque Tg mice only differed from Non-Tg mice on day 1 of cued testing, the spatial learning impairments demonstrated by the Tg mice at the early and late plaque ages were most likely not due to abnormalities in non-

cognitive aspects of the task (e.g., motivation, vision, or motor skills). Further, at these post-plaque ages, Tg and Non-Tg mice did not differ in other non-cognitive measures obtained during spatial testing, such as swimming speed, thigmotaxic behavior (wall-hugging), or floating, again suggesting that the spatial learning impairments demonstrated by the Tg mice at the post-plaque ages were most likely cognitive in nature.

In summary, although demonstrating normal short-term recognition memory, Tg CRND8 mice showed progressive, age-related impairments in the spatial Morris maze that paralleled age-related increases in cortical Aβ burden and production. Importantly, Tg CRND8 mice were not impaired in learning the Morris maze at a young pre-plaque age, suggesting that the behavioral impairments observed at the later ages were not simply related to the expression of the transgene, regardless of subsequent Aβ pathology. Furthermore, the spatial Morris maze impairments were observed in Tg CRND8 mice after, but not before, plaque deposition had begun, with the impairments becoming more severe as the mice aged and their neuropathology advanced. This suggests that the cognitive impairments observed in Tg CRND8 mice after 8 week of age were perhaps due to a cumulative effect of Aβ, formation of specific Aβ assemblies or oligomers [26] or cortical plaques being deposited. This is agreement with other studies that have reported progressive age-related cognitive impairments in transgenic AD mice that developed after plaque deposition had begun [1,5,6,8,10,11,23,28,29,30]. Further studies are needed to determine which aspect of Aβ neuropathology may be related to the cognitive impairments observed in Tg CRND8 mice. However, now that we have a clearer understanding of the relationship between Aβ neuropathology and cognition in Tg CRND8 mice, we can determine if therapies designed to reduce or eliminate Aβ production and prevent or delay the onset of plaque deposition will provide functional, cognitive benefits to these mice.

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