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Cognitive impairment in the Tg6590 transgenic rat model of Alzheimer's disease

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Abstract

Recently, interest in the rat as an animal model of Alzheimer's disease (AD) has been growing. We have previously described the Tg6590 transgenic rat line expressing the amyloid precursor protein containing the Swedish AD mutation (K670M/N671L) that shows early stages of Aβ deposition, predominantly in cerebrovascular blood vessels, after 15 months of age. Here we show that by the age of 9 months, that is long before the appearance of Aβ deposits, the Tg6590 rats exhibit deficits in the Morris water maze spatial navigation task and altered spontaneous behaviour in the open-field test. The levels of soluble Aβ were elevated both in the hippocampus and cortex of transgenic animals. Magnetic resonance imaging showed no major changes in the brains of transgenic animals, although they tended to have enlarged lateral ventricles when compared to control animals. The Tg6590 transgenic rat line should prove a suitable model of early AD for advanced studies including serial cerebrospinal fluid sampling, electrophysiology, neuroimaging or complex behavioural testing.

Keywords: Alzheimer's disease • transgenic • animal model • rat • behaviour

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. This devastating brain disorder is characterized by progressive cognitive decline, where memory of recent facts, spatial orientation, attention and executive functions are ones of the first affected [1]. The pathological hallmarks of the disease are abnormal accumulations of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFT) predominantly in the neocortex and hippocampus of AD patients. The main component of the plaques is β-amyloid (Aβ), a 39–43 amino-acid long proteolytic product of the amyloid precursor protein (APP). Aβ40 and Aβ42 are the most common amyloid isoforms. Neurofibrillary tangles are composed mainly of paired helical filaments of a hyperphosphorylated form of the microtubule-associated protein tau. Other features of AD neuropathology include synaptic and neuronal loss, as well as inflammatory processes.

Mutations in three genes, namely APP, presenilin 1 (PS1) and presenilin 2 (PS2), are responsible for up to one half of early onset cases of a hereditary form of AD [2] which shares the neuropathological characteristics of the more common, sporadic form of the disease. The mutations in these three proteins lead to altered production and secretion of Aβ. Therefore, APP processing is considered to be a key event in AD pathogenesis [3]. Since the identification of AD-causing mutations, numerous transgenic mouse models recapitulating many features of the disease have been developed (reviewed in [4]). These models have proven very valuable in dissecting the mechanisms involved in AD but there is still a need for a model better suited for more advanced studies, including serial cerebrospinal fluid sampling and complex behavioural testing. For these purposes, the rat offers several advantages over the mouse both due to its larger size and more complex behaviour repertoire [5]. However, to date, only a few transgenic

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rat models of AD have been reported. In 2004, a transgenic Fisher-344 rat expressing human APP carrying the Swedish AD mutation was described [6]. These rats showed low-levels of APPswe expression and no AD related pathology was found. The same year, a series of papers on a double-transgenic Wistar APP/PS1 rat model were published [7–10]. The rats expressed APP containing the Swedish and Indiana AD mutations as well as PS1 with an AD causing mutation. The main pathological feature of these animals was accumulation of intracellular Aβ in neurons of the hippocampus and cortex from the age of 6 months and followed by mild spatial memory impairment at 16 months of age. In 2007, a transgenic rat expressing human APP was reported, but no plaques or cognitive deficits were seen with age [11]. Recently, the generation of other multi-transgenic Sprague-Dawley rat lines was reported. These animals express APP containing the Swedish and London mutations, with or without mutated PS1 [12]. The double APP homozygous animals showed low amounts of extracellular Aβ deposits by the age of 17–18 months, whereas the triple transgenic homozygous rats displayed extracellular amyloid depositions at 7–9 months of age. Although the triple transgenic model displays extensive amyloid pathology, gross overexpression of multiple mutant transgenes may hinder deciphering the molecular steps leading to pathology.

We have previously reported the establishment of the Tg6590 transgenic rat [13] expressing APP with the Swedish AD mutation [14–15]. Beginning after 15 months of age Aβ deposition can be found predominantly in cerebrovascular blood vessels of the Tg6590 rats. Here, we report on behavioural testing, *in vivo* magnetic resonance imaging (MRI) and assessment of biochemical markers of significance for AD in the Tg6590 model.

Materials and methods

Animals

Male homozygous Tg6590 rats expressing human APP695 with the Swedish APP mutation under the control of the ubiquitin promoter and non-transgenic Sprague-Dawley (control) rats were used in all studies. All animals were bred at the Karolinska Institutet Animal Facility and housed two to four rats per Mac IV cage under controlled conditions of temperature (22°C) and humidity. Food pellets (BeeKay diet, B&K Universal AB, Sollentuna, Sweden) and tap water were available *ad libitum*. One week before behavioural testing the rats were handled daily to habituate with the experimenter. Behavioural tests were carried out during the light phase of 12-hr light/dark cycle. All experiments were conducted in accordance with the policies on animal ethics and welfare of the Southern Stockholm Animals' Ethics Committee.

Six Tg6590 animals and seven control animals at the age of 9 months were used for the behavioural studies. Five animals from each of these groups were later imaged by MRI (at the age of 11 months). After MRI analysis the animals were killed and the brains harvested for quantification of soluble Aβ and for Western blotting. Additionally, five Tg6590 and five age-matched control animals, 16 to 20 months old, were used for protein analysis by western blotting.

Spontaneous open-field test

The test apparatus consisted of a square grey PVC arena, 70 × 70 × 60 cm. The rats were placed individually in the centre of the arena, and their movements recorded for 60 min. using the Ethovision automated video tracking system (Noldus, The Netherlands). The behavioural parameters analysed were the distance moved and mean velocity of horizontal movement as well as rearing (defined as standing on hind limbs with the forelimbs in the air or against the wall of the arena) in peripheral and central zones of the testing arena, respectively. The test apparatus was cleaned with 70% ethanol and water after each animal.

Morris water-maze test

This test was used in order to assess spatial learning and memory in the Tg6590 rats. Briefly, the animal is released into a round swimming pool from randomly chosen positions and will naturally seek to escape the water. During the acquisition phase the location of a hidden platform is learned by referring to visual cues placed around the room [16]. For the current experiments the circular pool was made of grey PVC 140 cm in diameter and its walls were 50 cm high. Water temperature was maintained at 21 ± 2°C. A plastic transparent platform (9 × 9 cm) was placed approximately 0.7 cm below the water surface and 25 cm from the edge of the pool. Distal visual cues consisted of several wall posters, approximately 50–75 cm in size, cupboards and tracking equipment located in the testing room. One day before the start of the acquisition, the animals were habituated to the procedure during a 60-sec. swim trial. Rats were transferred to the testing room in a non-transparent cage to avoid visual orientation prior to release into the pool. Release points were balanced across four symmetrical positions on the pool perimeter. The position of the hidden platform remained fixed during the acquisition phase which lasted 5 days. Four trials of 60 sec. length with 30 sec. intertrial intervals were given per day. When a rat did not find the platform within 60 sec. it was placed on the platform and allowed to stay there for 30 sec. to assist its learning. In order to assess spatial memory, a probe trial was performed on the last day of the experiment about 40 min. after the last acquisition test. The platform was removed from the pool and the animal allowed to swim for 60 sec. A single visible platform test was performed 2 days later. For this test the platform was raised above the water surface, positioned in the centre of the circular pool and marked with a yellow flag. Rat behaviour in the Morris water maze test was recorded by an automated video-tracking system (Ethovision).

Magnetic resonance imaging

MRI was performed with a 4.7 T, 40 mm bore horizontal magnet (Bruker Biospec Avance 47/40, Bruker, Karlsruhe, Germany) fitted with a 12-cm inner diameter self-shielded gradient system (max. gradient strength 200 mT/m). A volume coil (Bruker) with 72-mm inner diameter was used for excitation and signal detection. Three-dimensional images were obtained using inversion recovery spin echo sequence with rapid acquisition with relaxation enhancement (RARE) [17]. The parameters were: repetition time (TR) 2.5667 msec., echo time (TE) 8.9 msec., RARE-factor 8 with RARE-maximum 4, inversion delay 500 msec., matrix size 64 × 64 × 128 and two averages. Total acquisition time was 1 hr 28 min. Field of view (FOV) for the 3D was 1.2 × 2.2 × 3 cm. Image reconstruction resulted in a resolution of 0.19 × 0.34 × 0.23 mm in dorso-ventral, left-right and rostro-caudal directions, respectively.

The rats were anesthetized with 1.5–2.0% isoflurane in air delivered *via* a mouth piece allowing spontaneous respiration. The rats were then positioned in supine position and the head fixed to an acrylic rig. Body temperature was recorded and maintained at about 37°C using a MRI-compatible air temperature control system.

MRI volumetric analysis

Three-dimensional MRI images were analysed using Amira 3.0 software (Mercury Computer Systems, Chelmsford, MA, USA). The different brain structures were segmented in accordance with the G. Paxinos and C. Watson atlas [18]. Structure volumes were estimated using stereological quantification based on Cavalieri's principle and point counting (reviewed in [19]). Choice of the first slice used for quantification was semi-random, as it was always the MRI slice where the given structure was first visible (in the direction from anterior to posterior for coronal slices and from dorsal to ventral for horizontal slices). The lateral ventricles were measured in each second contiguous coronal slice. Brain and cortex volumes were calculated from a total of 16 coronal slices (every other slice) between approximately +1.6 and –5.3 from Bregma point, characterized by continuous corpus callosum. Hippocampi were measured in all slices depicting it. The borders of the hippocampus were checked on both horizontal and coronal slices to ensure accuracy and calculations from both orthogonal planes were averaged. The total volumes were calculated by multiplying the estimated area by the slice thickness (0.23 mm). All measurements were performed twice by an investigator, who was blind with regards to genotype. The two measurements were performed with an interval of 3 weeks and the mean value calculated. The calculated volumes were divided by the brain volume of each animal, yielding a ratio that is adjusted to the animal's brain size.

Tissue preparation and Western blot

The right brain hemispheres were dissected and stored at –80°C until used. Tissues were homogenized in 20 volumes of buffer (PBS with 1% [w/v] CHAPS, 2.5 mM ethylenediaminetetraacetic acid, 2.5 mM EGTA, 2 mM Na_3VO_4, 60 mM NaF, 6 mM glycerol 2-phosphate) containing a protease inhibitor cocktail (1:400; Sigma-Aldrich). Homogenates were then gently mixed for 30 min. at 4°C, transferred to microcentrifuge tubes, and centrifuged for 90 min. at 17,000 × *g* to remove cellular debris. The supernatants were transferred to separate clean tubes, frozen on dry ice and stored at –70°C.

Protein content was determined using BCA protein assay (Pierce). Western blotting was performed as previously described [20]. Primary antibodies used in this study were: mouse anti-human APP 6E10 (Signet, diluted 1:2000), anti-Neuronal Nuclei (Neu N) (Chemicon, Billerica, MA, USA, 1:400); phospho-specific tau antibody PHF-1 (generously provided by P.Davies, 1:400); anti-total tau (tau-5) (Biosourse, Paisley, UK, 1:1000); phospho-specific tau antibody AT8 (Innogenetics, Gent, Belgium, 1:1000); rabbit anti-phospho tau antibodies tau ps396, ps404 and ps199 (BioSource, 1:1000) and polyclonal rabbit anti-synaptophysin (DakoCytomation, Glostrup, Denmark, 1:4000). The secondary antibodies purchased from Amersham, Uppsala, Sweden were diluted 1:2500. Optical density of protein bands were quantified using ImageJ software.

Aβ quantification

Total Aβ40 and Aβ42 in brain extracts were quantified by ELISA (Wako Chemicals USA, Inc., Richmond, VA, USA) following manufactures instructions. The frozen brains were homogenized in 10 volumes (w/v) of 0.2% diethylamine containing 50 mM NaCl (pH 10) and protease inhibitors (Roche Diagnostics, Mannheim, Germany), AEBSF, and 0.5% NP-40. Samples were incubated 30 min. on ice after brief sonication, then centrifuged at 20,000 × *g*, 4°C for 30 min. The resulting supernatant was retained as the soluble fraction and neutralized by addition of 10% 0.5 M Tris/HCl, pH 6.8. Samples were diluted 1:1 in 8 M urea, incubated 30 min. on ice and diluted fivefold before analysis.

Statistical analysis

For repeatedly measured parameters group differences were analysed by repeated measures ANOVA, unless stated otherwise. Tukey's HSD *post hoc* test was used whenever ANOVA showed significant main effects. MRI brain structure data were analysed using ANOVA. Non-parametric Mann–Whitney U-tests were used for comparing protein levels. The level of statistical significance was set at $P < 0.05$. All data are expressed as mean values ± S.E.M.

Results

At the start of behavioural testing the Tg6590 rats weighed significantly less than the age matched controls (486 ± 20 g and 550 ± 14 g, respectively, $P < 0.01$). Such a weight difference is also seen in several APP transgenic mouse models [21]. We have not observed any weight differences in younger animals, up to 4 months of age.

Behavioural studies

There were no significant differences between the spontaneous locomotor activity of transgenic and control animals over the 60-min. open-field exposure in terms of the total distance moved (Fig. 1A) or the speed of movement. There were also no differences in the frequency, duration spent and distance moved in the central and peripheral zones of the arena (data not shown). Over the whole 60 min. there was no significant group difference in rearing. However, during the first 40 min. in the open-field arena the Tg6590 rats reared significantly less than control animals, as revealed by the repeated measures ANOVA which showed a significant difference between the genotypes ($F[1, 11] = 10.5$, $P < 0.01$) and no interaction between time and genotype (Fig. 1B). The analysis of behaviour during a shorter than 60 min. time interval is interesting since rats are most likely to explore the environment while it is novel to them, gradually reducing rearing as they become habituated to the surroundings [22].

During the acquisition phase of the Morris water maze test (days 1–5) the Tg6590 rats spent significantly longer time trying to locate the platform as shown by a significant genotype effect on the escape latencies over time (ANOVA ($F[1, 11] = 31.7$, $P < 0.0005$)) (Fig. 2A). The Tg6590 rats swam significantly longer distances (path length) before locating the hidden platform (ANOVA

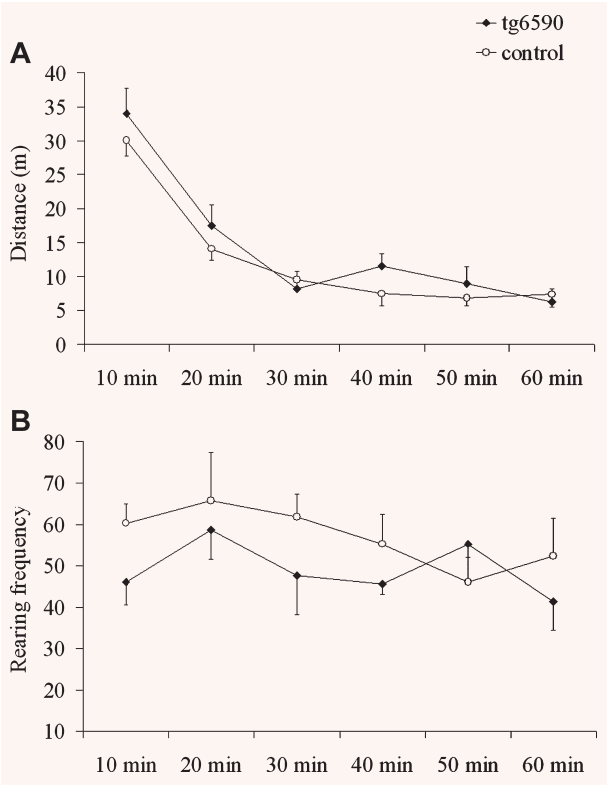

Fig. 1 Spontaneous behaviour of Tg6590 transgenic and control animals during 60 min. of open-field test. (**A**) Rearing activity. The group means ± S.E. in 10-min. bins. Significant difference was found in rearing activity of transgenic and control rats during the first 40 min. of the test ($P < 0.01$). (**B**) Locomotor (distance traversed) activity.

($F[1, 11] = 15.3$, $P < 0.005$)) (Fig. 2B). The swimming speed of Tg6590 was significantly lower than that of control rats (genotype effect in ANOVA ($F[1, 11] = 7.3$, $P < 0.05$)) (Fig. 2C). Tg6590 rats spent a significantly greater amount of time swimming next to the pool's walls (thigmotaxis) compared to control animals (genotype effect ANOVA ($F[1, 11] = 12.8$, $P < 0.005$)) (Fig. 2D). Neither swim speed, thigmotaxis nor body weight alone accounted for the impaired performance of the transgenic animals. When thigmotaxis, swim speed or body weight were entered as cofactors in the ANOVA for the escape latencies the significant difference in genotypes remained significant (genotype effect ANCOVA ($F[1, 10] = 7.9$, $P < 0.05$ and covariate thigmotaxis $P < 0.05$), genotype effect ANCOVA ($F[1, 10] = 13.9$, $P < 0.005$, and covariate swim speed $P =$ n.s.) and genotype effect ANCOVA ($F[1, 10] = 11.2$, $P < 0.05$, and covariate body weight $P =$ n.s.)).

On the last day of acquisition, after the last acquisition trial, the escape platform was removed and the animals were allowed to freely search the pool. During this probe trial, the Tg6590 rats performed significantly worse as determined by the analysis of five measures commonly used to assess memory for platform location/spatial memory: (*i*) latency to reach the platform area, (*ii*) frequency of platform crossings, (*iii*) distance swam, (*iv*) time spent and (*v*) frequency of entries into the target zone (Table 1). Only the control rats preferentially swam in the pool quadrant where the platform was previously placed (Fig. 3), which is indicative of good memory for the target quadrant ($P <$ 0.0001, Tukey's pairwise multiple comparison test comparing percentage search time in target quadrant against opposite, target quadrant against right and target quadrant against left following one-way ANOVA). The Tg6590 rats showed no preference in searching the pool quadrants.

No significant visual differences could be detected between the groups during the visible platform test as all rats swam similarly well to the raised platform.

MRI volumetric analysis

Two months after completion of behavioural testing, at the age of 11 months, five animals from each group were investigated by MRI. Volumetric analysis of the resulting scans showed no statistically significant group differences between absolute volumes of brain, hippocampus or ventricles in the Tg6590 and control animals (Table 2). Interestingly, two out of five transgenic rats had greatly enlarged ventricles (37% and 53% larger than mean control value) and the difference increased to 58% and 77%, respectively, when normalized to the animal's brain volume. These two rats did not perform differently than the other transgenics in the behavioural tests. No hippocampal or cortical atrophy was seen in the transgenic animals of this age; however, preliminary data suggest enlargement of lateral ventricles and a thinning of the cortex in older Tg6590 animals as compared to controls (16 months old, data not shown). Since only a limited number of older animals have been analysed (three in each group) additional animals need to be investigated before any definite conclusions can be drawn.

APP expression and Aβ levels

The expression of human APP protein in the brains of all Tg6590 rats was confirmed in this study by Western blot of brain homogenates using the human-specific 6E10 antibody. Total Aβ levels (rat and human) were measured in the 10 rats used in the behavioural study at 9 months of age and later imaged by MRI at 11 months of age. The Tg6590 rats had significantly higher levels of soluble Aβ40 and Aβ42 both in the hippocampus and the cortex, as compared to controls (Fig. 4). The hippocampal levels of Aβ40 and Aβ42 were increased by 67% and 65%, respectively, in the transgenic animals ($P < 0.01$), whereas cortical Aβ levels were elevated by 44% and 40%, respectively ($P < 0.01$). There were no differences in Aβ42/Aβ40 ratios between the animal groups. This was expected since the APPswe mutation affects β-secretase processing of APP resulting in an increase in both Aβ40 and Aβ42 [23].

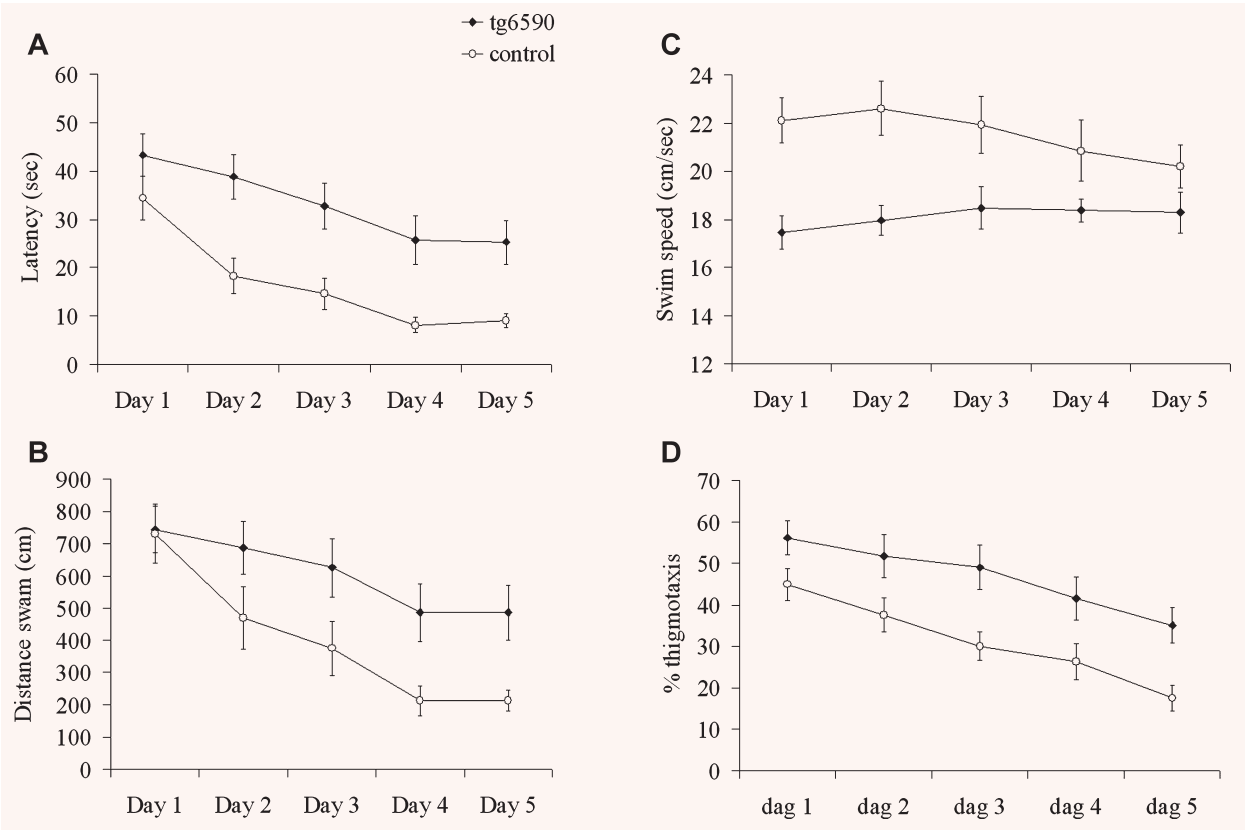

Fig. 2 Spatial learning in Tg6590 transgenic and control animals, assessed by Morris water maze. Blocks of four trials/day during 5 days of acquisition training shown as group means ± S.E. (**A**) Escape latency, $P < 0.0005$. (**B**) Speed of swimming, $P < 0.05$. (**C**) Swim path length, $P < 0.005$. (**D**) Thigmotaxis as percentage of total swim path spent in the periphery area, $P < 0.005$. Significant group differences by Tukey's *post hoc* following ANOVA with repeated measures.

Analysis of τ phosphorylation, synaptic and neuronal markers

We have previously reported an apparent increase in the phosphorylation of tau in old Tg6590 animals as detected by the PHF-1 antibody [13]. To follow up on this observation, in the present study the levels of different forms of tau were analysed by Western blots of brain homogenates. Within the two groups, τ phosphorylation at the double serine 396 and 404 site recognized by the PHF-1 antibody showed a large heterogeneity as previously reported. Overall, the transgenic animals tended to show higher phosphorylation at the PHF-1 recognition sites, but the differences were not statistically significant. None of the other phospho-specific antibodies used showed any differences in phosphorylation of τ in the Tg6590 and control rat brains. Total τ levels were similar in all animals. No differences in the levels of synaptic marker synaptophysin or neuronal marker NeuN could be detected by Western blotting in transgenic and control animals.

Discussion

Many transgenic mouse models of AD show behavioural impairments that are attributed to perturbations in components of the amyloid-processing pathway. The transgenic rat lines available so far show lower expression levels of the APP transgene than mouse AD models and only mild or no Aβ deposition in the brain. The only exception is the triple-transgenic homozygous rat carrying two mutant APP constructs and one mutated PS1, which shows extensive extracellular amyloid deposits [12]. This rat line has however been shown to be prone to premature death due to such health problems as chronic kidney disease, hypertension and immunosuppression [24], which sets limitations on its use as an AD research model.

Recently more attention has been focused on the effects of soluble forms of Aβ oligomers on cognition, as it has been shown that there is no simple correlation between plaque load in AD and dementia [25–26]. Long-term effects of Aβ42 immunization

Table 1 Morris water maze. probe trial with the platform removed after the last acquisition training trial, 9 months old animals

Group	Latency	Crossings	Distance in Targeted quadrant	Duration in Targeted zone	Frequency entries Targeted zone
Tg6590	38.74 sec ± 10.24*	1.5 ± 0.76*	296.34 cm ± 46.87*	1.57 sec ± 0.8*	1.33 ± 0.61*
control	8.78 sec ± 3.4	3.86 ± 0.55	463.29 cm ± 25.19	4.91 sec ± 0.82	4.86 ± 0.74
Group differences	$F(1,11) = 8.78$ $p < 0.01$	$F(1,11) = 6.5$ $p < 0.05$	$F(1,11) = 10.7$ $p < 0.01$	$F(1,11) = 8.4$ $p < 0.01$	$F(1,11) = 12.9$ $p < 0.005$

Mean ± S.E. *statistically significant differences for Tg6590 vs. control animals by ANOVA.

in AD patients showed, in seven of eight immunized patients, at post mortem investigation that although plaques were removed the patients had no improved survival and they all progressed to severe dementia. This indicates that removal of plaques is not sufficient to prevent progression of the clinical symptoms [27].

The elevation of soluble Aβ in Tg6590 is less than that seen in the APPswe transgenic mouse models Tg2576 and TgAPP23, but more than in the TgAPPswe Fisher-344 rat. Whereas the TgAPPswe Fisher-344 rats showed attenuated age-dependent memory decline, our 9-month-old Tg6590 transgenic animals exhibit learning and memory deficits in a spatial navigation task and altered exploratory behaviour in the open-field test.

The Tg6590 rats performed worse both during the acquisition phase of the Morris water maze test and the probe trial retention test. The slower swim speed of the Tg6590 rats could indicate motor deficits, which were not evident in the open-field test. On the other hand there was no significant difference in swim speed during the visible platform test. These results suggest that any motor impairments may only be detectable during physically demanding tasks and will require detailed behavioural testing in order to clarify this issue. The transgenic animals also showed more thigmotaxic swimming (amount of time spent circling near the edge of the pool), which could be a sign of anxiety [28] or lack of focus on learning the task. Neither the swim speed nor thigmotaxis affected the results of the probe trial expressed as the percentage of time spent in each pool quadrant, which clearly indicated blunted recall in the transgenic animals. The results presented here showing impaired spatial learning and memory in the Tg6590 rats similar to that shown for the recently published triple-transgenic homozygous rats [29]. Consistent with findings in several APP mouse models and our own rat line, the performance in Morris water maze task is affected before the appearance of amyloid plaques, supporting the hypothesis that soluble forms of Aβ are responsible for the cognitive deterioration.

In the open-field test, a much less physically demanding task, there was no difference in the speed or distance moved between the two groups. However, the Tg6590 rats displayed significantly less rearing activity during the initial phase of the task. Rearing activity of rodents in a novel environment has been interpreted as

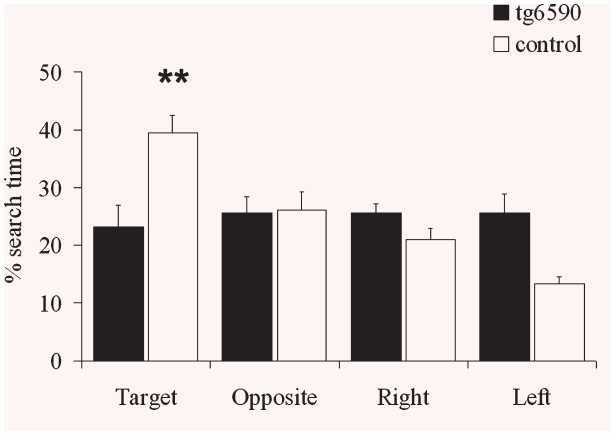

Fig. 3 Preference for pool quadrant during probe trial in Morris water maze. Mean percentage of time ± S.E. spent in each quadrant by Tg6590 transgenic and control animals. $^{**}P < 0.0001$ by Tukey's pairwise multiple test comparing percentage search time in target quadrant *versus* opposite; target *versus* right; target *versus* left.

an orienting behaviour important for gathering information but also a measure of the animal's motivational state and general arousal level [30]. Further behavioural analysis of the Tg6590 rats would show whether the results are indicative of attention deficits, which have been shown to be characteristic for human patients during early stages of AD [31]. It is interesting to note that differences in rearing in an open-field test have also been reported for the Tg 2576 mouse model which over expresses the human APP with the Swedish double mutation, and are age dependent [32].

MRI showed no substantial structural alternations in the Tg6590 rats used in behavioural testing, although overall the transgenic animals tended to have enlarged ventricles as compared to controls. We could not detect any hippocampal or cortical atrophy in the 11-month-old animals, and have only used a few older rats available for imaging. Nevertheless, preliminary data suggest hippocampal and cortical diminution in 16-month-old animals. No synaptic or neuronal loss has been detected by Western blotting, but this method will only show gross changes. But,

Table 2 Estimated volumes of brain structures of 11 month old animals

Group	Whole brain*	Cortex*	Hippocampus	Ventricles
Tg6590	953 ± 39.9 µl	283 ± 13.2 µl (29.7 ± 0.6)	91.3 ± 4.3 µl (9.6 ± 0.1)	39.6 ± 6.5 µl (4.2 ± 0.9)
control	999 ± 13.4 µl	296 ± 4.6 µl (29.7 ± 0.5)	94.4 ± 3.6 µl (9.4 ± 0.3)	37.8 ± 3.7 µl (3.8 ± 0.4)

only the fragment between +1.6 and -5.3 from Bregma point was calculated since it was difficult to distinguish structures beyond this region. 100 x ratios of calculated volumes divided by brain volume of each animal are depicted in brackets.

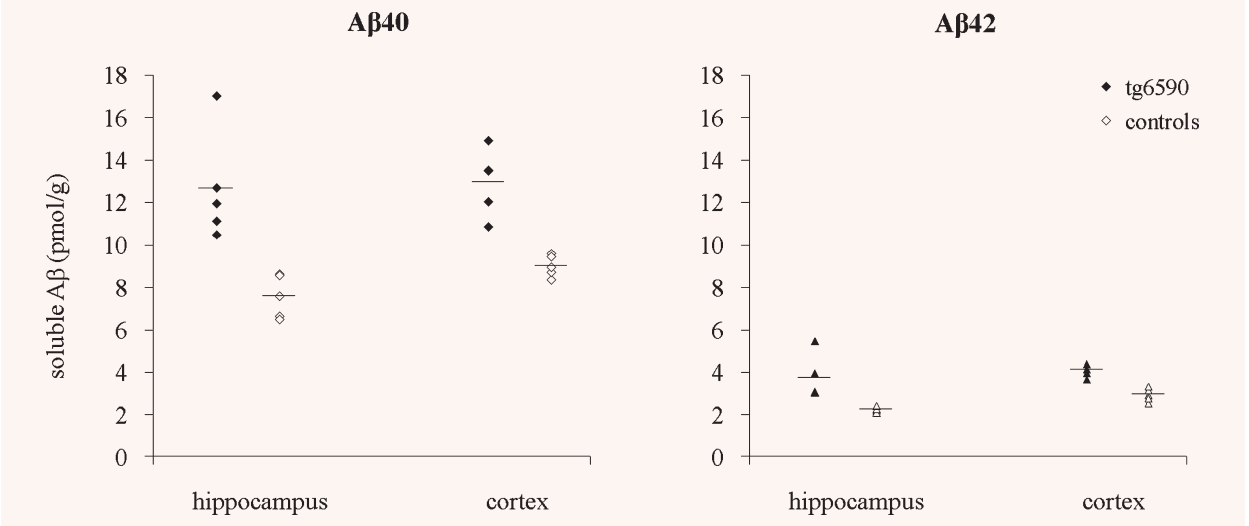

Fig. 4 Aβ40 and Aβ42 levels in the hippocampus and cortex of 11-month-old transgenic Tg6590 and control animals (pmol/g brain tissue).

neurodegeneration has also been shown to be mild or missing in many APP and APP/PS mice models [4], which could at least partly be explained by the neurotrophic effects of secreted APPα in transgenic animals.

The death of neurons is by no means a prerequisite for memory loss as has been demonstrated by the transient effect of Aβ*56 oligomers on performance of young healthy rats injected with this peptide [33]. It is also well known that AD patients may have striking fluctuations in functional abilities even within the same day, which strongly implies that at least some of the neurological impairment is due to a dysfunction of neuronal networks rather than loss of neurons [34]. We have previously shown that primary neurons derived from the Tg6590 rats have increased intracellular calcium levels and altered calcium signalling which is indicative of aberrant neuronal activation [35–36] and could underlie the observed memory impairment.

Beside amyloid plaques, the other key pathological feature of AD is abundance of intracellular neurofibrillary tangles composed mainly of paired helical filaments of a hyperphosphorylated form of the microtubule-associated protein tau. Due to high heterogeneity in the level of tau phosphorylation at the PHF-1 phosphosite in this study, the difference between the two groups of animals was not statistically significant. The individual variation between the animals is consistent with the results obtained using this antibody in the double APP/PS rat model [9].

In summary, our data support the hypothesis that cognitive deterioration in AD can be independent of amyloid deposition into plaques. Our single transgenic APPswe Tg6590 rats, showing increased levels of Aβ40 and Aβ42 in the hippocampus and cortex might prove suitable for testing new therapeutic strategies aimed at improving memory in AD patients at the early stages of the disease.

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