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In vivo effects of APP are not exacerbated by BACE2 co-overexpression: behavioural characterization of a double transgenic mouse model

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Abstract

Down syndrome, the most common genetic disorder leading to mental retardation, is caused by the presence of all or part of an extra copy of chromosome 21. At relatively early ages, Down syndrome patients develop progressive formation and extracellular aggregation of amyloid-β peptide, considered as one of the causal factors for the pathogenesis of Alzheimer's disease. In Down syndrome, this neuropathological hallmark has been attributed to the overexpression of *APP* that maps to HSA21. Since *BACE2* maps to chromosome 21 and is homologous to *BACE1*, a β-secretase involved in the amiloidogenic pathway of APP proteolysis, it has been hypothesized that the co-overexpression of both genes could contribute to Alzheimer disease neuropathology present in Down syndrome. The aim of the present study has been to analyze the impact of the co-overexpression of *BACE2* and *APP*, using a double transgenic murine model. TgBACE2-APP mice did not have any neurological or sensorimotor alterations, but an increased in total horizontal distance travelled and number of rearing behaviour was reported. Both genes overexpression do not lead to anxiety-like behaviour or age-dependent cognitive dysfunction. Interestingly, the co-overexpression of *BACE2* and *APP* do not increase amyloid-β peptide concentration in brain.

Introduction

Down syndrome (DS) or trisomy 21 is a clinically heterogeneous disorder which shows hypotonia in the newborn period, followed by developmental delay, mental retardation and Alzheimer's disease (AD) type neuropathology by age 30-40 years {Holtzman, 1996 #93; Wisniewski, 1985 #94}. However, the precise mechanism(s) by which trisomy 21 leads to either developmental or the early-onset of AD-like neuropathology remains to be elucidated.

A segmental trisomy mouse model of DS, Ts65Dn, which possesses a third copy of MMU16 spanning from *App* to *Mx1*, exhibits behavioural and cognitive abnormalities during early postnatal development that persist throughout adulthood and age-related neurodegeneration {Galdzicki, 2001 #95; Holtzman, 1996 #93; Coussons-Read, 1996 #75; Reeves, 1995 #54; Salehi, 2006 #96}. Extracellular aggregation of amyloid-β (Aβ) peptide, one of the causal factors for the pathogenesis of AD {Yan, 2001 #24}, is common in DS patients at relatively early ages and has been attributed to the over-expression of amyloid precursor protein (*APP*) {Murphy, 1990 #7}, a large type I transmembrane glycoprotein precursor that maps to human chromosome 21 (HSA21).

Aβ peptide is generated by the endoproteolytic processing of APP, when a β-secretase cleaves APP to generate APPsβ, a soluble N-terminal fragment, and a C-terminal fragment (C99). This C99 fragment is cleaved by a γ-secretase to form the mature Aβ peptide comprising 39-42 amino acids {Mattson, 2004 #39}. It has been observed an increased accumulation of the C99 fragment in the brain of DS individuals {Busciglio, 2002 #82; Sun, 2006 #12}, suggesting that abnormal processing at the APP β-site might be involved in DS.

It has been shown that the β site APP cleaving enzyme 1 (BACE1) protease is one of principal enzyme in this pathway {Sambamurti, 2004 #33; Vassar, 1999 #1; Hussain, 1999 #2}. Since *BACE1* has a paralogous gene in vertebrates, *BACE2*, which in humans maps to HSA21 at 21q22.3 in the DS critical region {Acquati, 2000 #8; Solans, 2000 #29} it has been speculated that BACE2 co-overexpression with APP would promote the early appearance of amyloid plaques in DS patients.

However, there has been an intense debate about BACE2 function. Some *in vitro* studies showed that BACE2 cleaves APP at the β-secretase site {Farzan, 2000 #30; Hussain, 2000 #3}, but others that cleaves at the α-secretase site {Yan, 2001 #24; Basi, 2003 #48} or at a novel site named θ-secretase site {Sun, 2006 #12}. Recently, an *in vivo* study has suggested that BACE2 is not involved in the amyloidogenic pathway, cognitive dysfunction or cholinergic degeneration observed in elderly people with DS but has reported that TgBACE2 animals showed increased anxiety-like behaviour along with increased numbers of noradrenergic neurons in the locus coeruleus {Azkona, 2009 #100}.

The aim of the present study has been to analyze the impact of the co-overexpression of *BACE2* and *APP* during the lifetime using a double transgenic mouse model (TgBACE2-APP).

Materials and Methods

Animals

To generate transgenic mice co-overexpressing *BACE2* and *APP*, we crossed previously characterized heterozygous TgBACE2 females {Azkona, 2009 #100} with heterozygous TgAPP males{Lamb, 1993 #97}, both generated on C57BL/6J x SJL F1 (B6/SJLF1/J) genetic background. Wild-type, TgBACE2, TgAPP and TgBACE2-APP littermates were housed in standard macrolon cages (4-5 animals per cage, 40 x 25 x 20 cm) with freely available food and water in standard environmental conditions (constant humidity and temperature of 22 ± 1ºC) and a 12 h light/dark cycle (lights on at 7:00 a.m.). All animal procedures were approved by the local ethical committee (CEEA-IMIM and CEEA-PRBB), and met the guidelines of the local (law 32/2007) and European regulations (EU directive nº 86/609, EU decree 2001-486) and the Standards for Use of Laboratory Animals nº A5388-01 (NIH). The CRG is authorized to work with genetically modified organisms (A/ES/05/I-13 and A/ES/05/14) and the experimenters hold the official accreditation (law 32/2007).

Western blotting

Four adult animals (4 months) for each genotype were sacrificed, brains rapidly removed and frozen. Tissues were homogenized in lysis buffer (10 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1 mM $MgCl_2$, phosphate-buffered saline (PBS) 0.2% Triton X-100 and a protease inhibitor cocktail (Roche, Mannheim, Germany). After clearance of the lysates by centrifugation (1400xg, 20 min at 4ºC), protein quantification was performed following the BCA Protein Assay Reagent (Pierce, Rockford, IL, USA) protocol. Western blot analysis was performed using 50 µg of

protein resolved on a 10% SDS-PAGE and electro-blotting onto nitrocellulose membranes (Hybond-C, Amersham Pharmacia Biotech, Freiburg, Germany). Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline including 0.1% Tween-20 (TBS-T) and incubated with the primary antibodies in 5% non-fat dry milk in TBS-T overnight at 4ºC. The following antibodies were used as primary antibodies: goat anti-Bace2 antibody (1:500; D-20; Santa Cruz, Heidelberg, Germany), rabbit anti-App antibody (N-Terminal, 1:1000; Sigma, Sant Louis, MO, USA), and anti-actin antibody (1:2000; Sigma, St Louis, MO, USA). Incubation with horseradish peroxidase (HRP)-conjugated anti-goat, anti-rabbit or anti-mouse IgG (Pierce, Rockford, IL, USA), followed by enhanced chemiluminescence (ECL, Pierce, Rockord, IL, USA) assay allowed detection. Quantification was made by densitometric analysis of non-saturated films (Quantity One image software).

Quantification of $A\beta_{1-40}$ and $A\beta_{1-42}$

$A\beta_{1-40}$ and $A\beta_{1-42}$ peptide levels were determined using an enzyme-liked immunoadsorbent assay (ELISA; Covance, Dedham, MA, USA) using soluble extracts from whole brain, cerebral cortex and hippocampus of 4 wild-type and 4 TgBACE2-APP mice per genotype and age, and following the manufacturer's instructions.

Preweaning behaviour

Fifty one animals of the four genotypes, wild-type (n = 12) and TgBACE2-APP (n = 16), from seven different litters were studied. All the pregnant dams were allowed to deliver spontaneously. The day of delivery was designated as PD1 of age of the neonates (erroneous estimates on time of birth = ± 6h). On delivery, the litter size of each dam was recorded and each pup was checked for gross abnormalities. The pups

were individually marked with India ink on PD1 and were nursed by their natural dams until weaning. During the testing protocol whole litters were separated from the dams and maintained for 30 min in a warmed environment. Neurobehavioral development on PD1-21 was assessed by daily testing of the pups according to the procedure of Dierssen {Dierssen, 2002 #98}.

Behavioural analysis in adult and old animals

To check behavioural genotype-associated changes at two different ages, we studied adult (4 months) wild-type (n = 14) and TgBACE2-APP (n = 10) and old (18 months) wild-type (n = 10) and TgBACE2-APP (n = 8) male mice. The behavioural characterization consisted in a neurological test battery, analysis of the locomotor activity, anxiety-like behaviour and cognitive profile (see below). The experiments were performed with an increasing gradient of stress to avoid interference in the results.

Neurological Assessment

SHIRPA primary screen is a comprehensive semiquantitative routine testing protocol to identify and characterize phenotype impairments during which 40 separate measurements are recorded for each animal, including somatometry {Rogers, 1997 #58}.

Locomotor Activity

Locomotor activity was measured by using actimetry boxes (45 x 45 cm; Panlab SL, Spain) contained in a soundproof rack mount cabinet. Horizontal movements were monitored by means of an infrared beams grid and used as an index of locomotor activity (counts). Counts were integrated every hour and added to obtain total locomotor

activity for a 24 h period maintaining the 12:12 h light-dark schedule. The measured parameters in the present study were total distance travelled by the animals (cm) and mean velocity (cm/sec).

Open Field Test

The open field was a white melamine box (70 x 70 x 50 cm high) divided in two zones, centre and periphery, being the centre more anxiogenic, and under high intensity light levels (300 Lux). At the beginning of the test session, mice were left in the periphery of the apparatus and during 5 min we measured and analyzed the latency to cross from the periphery to the centre, total distance travelled, average speed, and time spent in various parts of the field (e.g. the border areas vs. the open, central area).

Light and Dark Box

We used a box consisting of a small (15 × 20 × 25 cm) compartment with black walls and black floor dimly illuminated (25 Lux), connected by a 4 cm long tunnel to a large compartment (30 × 20 × 25 cm) with white walls and a white floor, intensely lit (500 Lux). Mice were individually placed in the dark compartment facing the tunnel at the beginning of the 5 min observation session. Number entries to light and dark zones, and in the tunnel connecting both zones, and time spent in each were recorded, as well as the latency to the first visit to the light zone.

Elevated Plus Maze

The elevated plus maze consisted of a black Plexiglas apparatus with four arms (29 cm long x 5 cm wide) set in cross from a central square (5 cm x 5 cm). Two opposite arms were delimited by vertical walls (closed arms), and the other two had

unprotected edges (open arms). The maze was elevated 40 cm above the ground under dim light (100 Lux). At the beginning of the 5 min observation session, each mouse was placed in the central zone, facing one of the open arms. The total numbers of visits to the closed and open arms, and the time spent in open and closed arms were recorded. An arm visit was recorded when the mouse moved all four paws into the arm.

Morris Water Maze

The swimming pool, 120 cm diameter and 0.5 m height, was filled with water (24 ± 1ºC) made opaque with non-toxic white paint and several fixed room cues were constantly visible from the pool. In the first day (training session), the escape platform (15 cm diameter, 24 cm height) was visible and placed in the centre of the pool to train the animal to escape from water. Four training trials were performed, entering the mice for four starting positions (north, south, east or west). During the following 5 days (Days 2-6) animals were tested for place learning acquisition with the escape platform located in the middle of the northeast quadrant, 1 cm below water surface. Four trials per day were performed (30 min inter-trial interval), mice entering randomly from each one of the starting positions and allowed to swim until they located the platform. Mice failing to find the platform within 60 sec were placed on it and left there for 20 sec, as the successful animals. On the day 7^{th}, we removed the platform from the pool, and four probe trials (60 sec) were performed, in which the time spent and distance traveled in the trained and non-trained quadrants were recorded. Finally, a session with a cued visible platform, situated in the centre of the pool and 1 cm above the water surface, was carried out. All the trials were recorded and traced with an Image tracking system (SMART, Panlab SL, Barcelona, Spain) connected to a video camera placed above the pool.

Passive Avoidance

We used a step-down passive avoidance test, which consisted of a transparent Plexiglas circular cage (40 cm in height, 30 cm in diameter) with a grid floor and a circular platform (4 cm diameter) in the center. During the training session, animals were placed on the platform and their latency to step down with all four paws was measured. Immediately after stepping down on the grid, animals received an electric shock (0.6 mA, 2 sec). Retention test sessions were carried out 24 h (short-term) and 7 days after training (long-term). Step-down latency was used as a measure of memory retention. A cut-off time of 300 sec was set.

Data analysis

Statistical analysis was performed using the software SPSS 12.0. Data were summarized as mean ± standard error of mean (S.E.M.) when normality might be assumed. For the analysis of the Western blot and ELISA results, Student's t analysis was used. Between-group comparisons were analyzed using one-way analysis of variance (ANOVA) or a two-way ANOVA with genotype and age as factors and with acquisitions trials as a repeated measure and significant effects were analyzed *post hoc* using Bonferroni test. The Passive avoidance test was analyzed using Mann-Withney U non-parametric test. In all tests, a difference was considered to be significant if the obtained probability value was $P < 0.05$.

Results

Generation and preweaning behaviour of TgBACE2-APP mice

TgBACE2-APP mice were generated by crossing TgBACE2 females with TgAPP males, and transgenic pups were born at the expected frequency (wild-type = 23.2%, TgBACE2 = 28.6%, TgAPP= 19.6% and TgBACE2-APP = 28.6%). Transgene expression at the protein level was confirmed by Western blot. As expected, an increase in App protein levels was observed in the brains of both TgAPP (43.1 ± 14.6%), and TgBACE2-APP mice (78.1 ± 32.2%) compared to wild-type littermates (Fig. 1A). Likewise, TgBACE2 mice showed an increase in Bace2 protein levels in comparison with wild-type littermates (30.7 ± 8.1%, P = 0.07). Moreover, in TgBACE2-APP animals the increase of Bace2 protein was significant in double transgenic mice when compared with wild-type (126 ± 14.4%, P = 0.001), and single transgenic BACE2 mice (95.9 ± 14.4%, P = 0.012; Fig. 1B). Interestingly, TgAPP mice did not show Bace2 overexpression and TgBACE2 did not show App over-expression (data not shown).

Studying the preweaning behaviour, statistical analysis found no significant differences between wild-type and TgBACE2-APP mice in the somatometry, developmental landmarks, neurobehavioral development or in the neuromotor development (Fig. 2).

General characterization of adult and old TgBACE2-APP mice

Physical characteristics such as body weight and the presence of bald patches and appearance of behavioural anomalies in the home cages were registered systematically with no differences between genotypes. Neurological assessment using modified Primary SHIRPA protocol revealed that spontaneous activity or sensory,

motor and autonomic functions were not affected by *BACE2* and *APP* co-overexpression in adult and old mice (Supplementary Table 1).

The study of the total locomotor activity revealed a significant increase in distance travelled ($F_{(1,23)}$ = 7.59, P = 0.012) and, mean velocity ($F_{(1,23)}$ = 5.37, P = 0.031) in adult TgBACE2-APP comparing to wild-type animals. On the other hand, decreases in distance travelled and mean velocity was observed due to age in studied genotypes, but no genotype-dependent differences were found between them (Fig. 3).

In the open field test, TgBACE2-APP (adult, $F_{(1,23)}$ = 5.11, P = 0.034 and old, $F_{(1,17)}$ = 4.67, P = 0.046) mice showed increased vertical activity comparing to their respective wild-type controls (Fig. 4A) but both horizontal distance travelled and mean speed were unaffected (data not shown). In the light and dark box, the latency to cross from dark to light compartment was no different between genotypes (Fig. 4C), and all studied groups spent similar time in the dark box (data not shown). In the elevated plus maze, there were no differences among genotypes in the time spent in the protected arm, but the age-related increase in anxiety-like behaviour was significantly more important in TgBACE2-APP ($F_{(1,17)}$ = 19.68, P = 0.001; Fig. 4D).

Cognitive characterization in adult and old TgBACE2-APP

Spatial learning was examined using the Morris water maze. In the training session, no genotype-related differences were observed in both studied ages. Along the 5 consecutive acquisition sessions, all animals learned the task (two-way ANOVA repeated measure; acquisition effect: adult: $F_{(4,23)}$ = 44.3, P = 0.0001, old: $F_{(4,17)}$ = 42.8, P = 0.0001), without genotype-related differences (Fig. 5A). In the removal session (Fig. 5B) a significant increase in the percentage of time spent in the trained quadrant (northeast) comparing to non trained quadrants was observed (two-way ANOVA, $F_{(1,41)}$

= 68.4, P = 0.001) but no genotype or age significant differences were observed. Finally, in the cued session, no significant motor or motivational problems were detected (Fig. 5A).

In the passive avoidance, similar step-down latencies were observed in all four genotypes at studied ages, indicating that the co-overexpression of *BACE2* and *APP* do not affect neither the short-term nor long-term memories (Fig. 6A and B).

Aβ peptides concentration in adult and old TgBACE2-APP

To determine whether the co-overexpression of *BACE2* and *APP* led to an increase of Aβ peptides we measured the concentration of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ by ELISA in wild-type and TgBACE2-APP adult and old animals. The results showed no differences in the amount of any of the peptides in the double transgenic mice of either age when compared to control littermates in the whole brain (data not shown), and the cerebral cortex and the hippocampus, the more affected brain areas in DS and AD (Table 1).

Discussion

The phenotype of DS is thought to result from the triplication of a gene or genes located on the HSA21. The present study analyzes the co-overexpression of two HSA21 genes, *BACE2* and *APP*, in a background free from dosage effects of other HSA21 and we have focused our work in the central nervous system to gain an insight into the involvement of the co-overexpression of both genes in the AD-like neuropathology observed in elderly people with DS.

The preweaning characterization showed that the sensori-motor delay observed in DS patients (Holtzman, 1996 #93) does not seem to be dependent on *BACE2* and *APP* overexpression. Similarly, none of the adult and old double transgenic mice exhibited any sensory or motor deficits which could interfere with performance in the cognitive tasks, although TgBACE2-APP showed an increased locomotor activity. Interestingly, altered activity has been previously described for some TgAPP models Harris-Cerruti, 2004 #101} and DS mouse models {Sago, 1998 #102. Previous results showed that TgBACE2 {Azkona, 2009 #100} and TgAPP {Harris-Cerruti, 2004 #101}, however, in the experiments we performed TgBACE2-APP mice did not show any increase in anxiety-like behaviour.

Finally, TgBACE2-APP mice did not show any impairment in visual-spatial learning and memory, or in recent memory in the passive avoidance test, indicating that co-overexpression of *BACE2* and *APP* is not able to develop the cognitive phenotype observed in DS, AD patients and DS mouse models. In fact, our ELISA results demonstrate that neuronal *BACE2* and *APP* co-overexpression does not affect $A\beta_{1-40}$ or $A\beta_{1-42}$ production in the cerebral cortex or the hippocampus. In this way, our results

confirmed in vitro and in vivo studies reporting that BACE2 is not involved in the APP amyloidogenic pathway (Sun *et al.* 2006; Azkona, 2009 #100).

In conclusion, the present study demonstrates that *in vivo* co-overexpression of both genes is not involved in the age-dependent cognitive impairment; besides aged TgBACE2-APP mice do not show increased Aβ production.

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References

Figure Legends

Figure 1. Transgene expression by Western blot. As expected **A.** App expression in *APP* overexpressing animals and **B.** Bace2 expression in *BACE2* overexpressing animals was higher than wild-type (WT) animals. Upper panels: representative images from each genotype. Lower panels: representation of the densitrometric quantification expressed as means of percentage from control ± SEM. ** $P < 0.01$.

Figure 2. Preweaning behaviour. No significant differences between genotypes were found. **A.** Weight (g) of mouse pups during the preweaning period. **B.** Achievement of mature responses in reflexological and behavioural tasks. **C.** Developmental physical landmarks. **D.** Pivoting activity. **E.** Walking activity. **F.** Homing test. (PD = Postnatal day). Data are expressed as mean ± S.E.M.

Figure 3. Locomotor activity. BACE2-APP co-overexpressing adult animals showed a significant increase in **A.** total distance travelled and **B.** velocity in the actimetry box during 24 h comparing to wild-type (WT) animals. No genotype-dependent differences were found between old groups. Data are expressed as mean ± S.E.M. * $P < 0.05$.

Figure 4. Open field test. A. TgBACE2-APP animals showed increased number of rearing movements comparing to their respective wild-type (WT) controls. **B.** No differences were observed in the latency to cross from periphery to centre. **Light and dark box. C.** No differences were observed in the latency to cross from dark to light compartment. **Elevated plus maze. D.** No differences were observed among genotypes in the time spent in the protected arm, but and the age-related increase was significantly

more important in TgBACE2-APP mice. Data are expressed as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$.

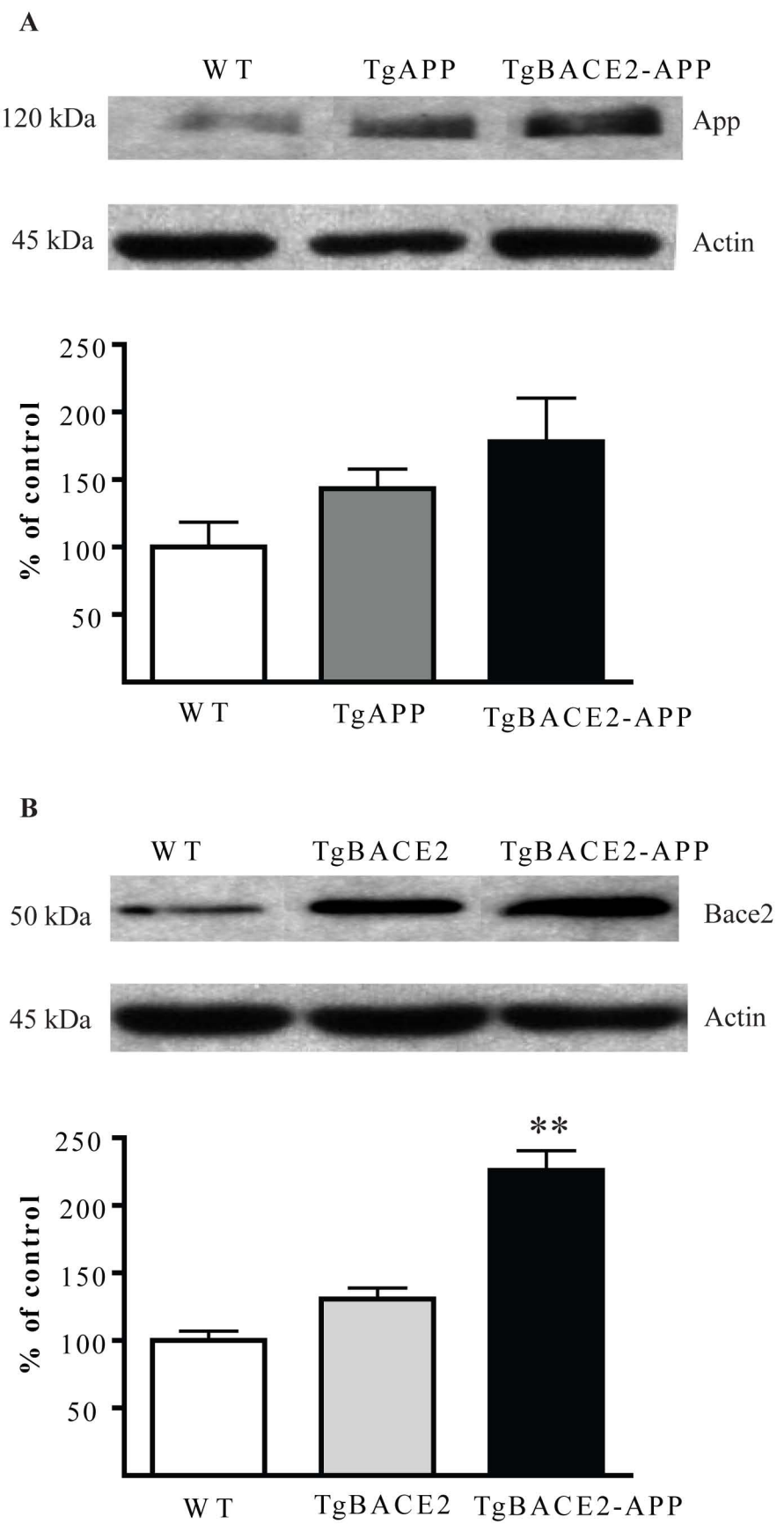
Figure 5. Morris Water Maze. (a) Escape latencies along the different sessions of the test. No differences between genotypes were observed at either studied ages and the age-dependent memory loss was similar in both genotypes (T = training; A = acquisition). **(b)** In the removal session no differences were observed between genotypes in the preference for the trained quadrant (NW = northwest, trained quadrant; NE = northeast; SW = southwest; SE = southeast). Data are expressed as mean ± S.E.M.

Figure 6. Step-down passive avoidance. No differences were observed between genotypes in the latency to exit the platform in the studied ages. Data are shown as the median and interquartile rates.

Table 1. **ELISA quantification of $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in cerebral cortex and hippocampus of wild type and TgBACE2-APP animals**

	CEREBRAL CORTEX				HIPPOCAMPUS			
	$A\beta_{1-40}$		$A\beta_{1-42}$		$A\beta_{1-40}$		$A\beta_{1-42}$	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Wild-type	0.9 ± 0.2	1.0 ± 0.7	1.0 ± 0.4	1.4 ± 0.5	1.1 ± 0.4	1.3 ± 0.3	0.7 ± 0.8	1.02 ± 0.9
TgBACE2-APP	1.0 ± 0.3	0.9 ± 0.5	1.2 ± 0.7	1.3 ± 0.3	1.1 ± 0.6	1.3 ± 0.5	0.7 ± 0.6	1.07 ± 0.8

No differences were observed between genotypes and ages. N = 4 per genotype. Data (pg/ml) are expressed as means ± S.E.M.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App
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Actin
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200
150
100
50
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TgBACE2-APP
B
WT
TgBACE2
TgBACE2-APP
50 kDa
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45 kDa
Actin
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% of control
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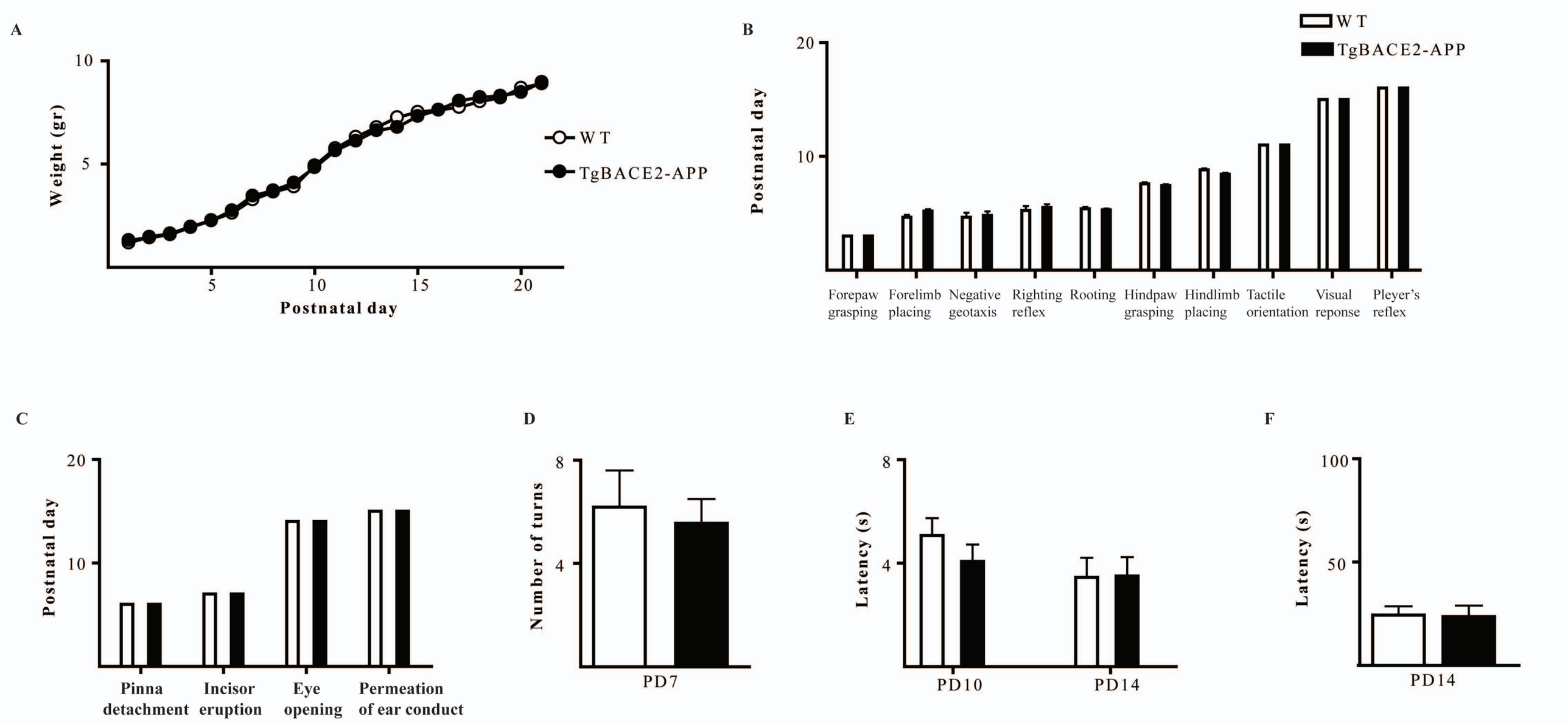
TgBACE2-APP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5
5
10
15
20
Postnatal day
WT
TgBACE2-APP
B
WT
TgBACE2-APP
Postnatal day
20
10
Forepaw grasping
Forelimb placing
Negative geotaxis
Righting reflex
Rooting
Hindpaw grasping
Hindlimb placing
Tactile orientation
Visual reponse
Pleyer's reflex
C
Postnatal day
20
10
Pinna detachment
Incisor eruption
Eye opening
Permeation of ear conduct
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PD10
PD14
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Latency (s)
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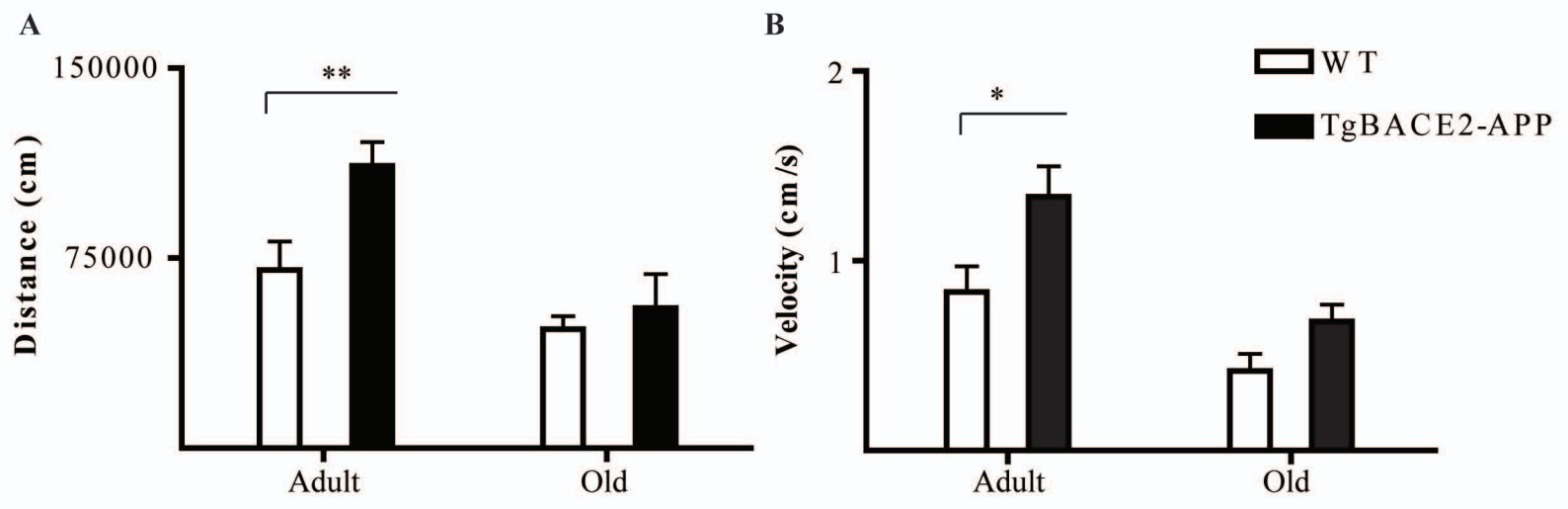
PD14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Velocity (cm/s)
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Adult
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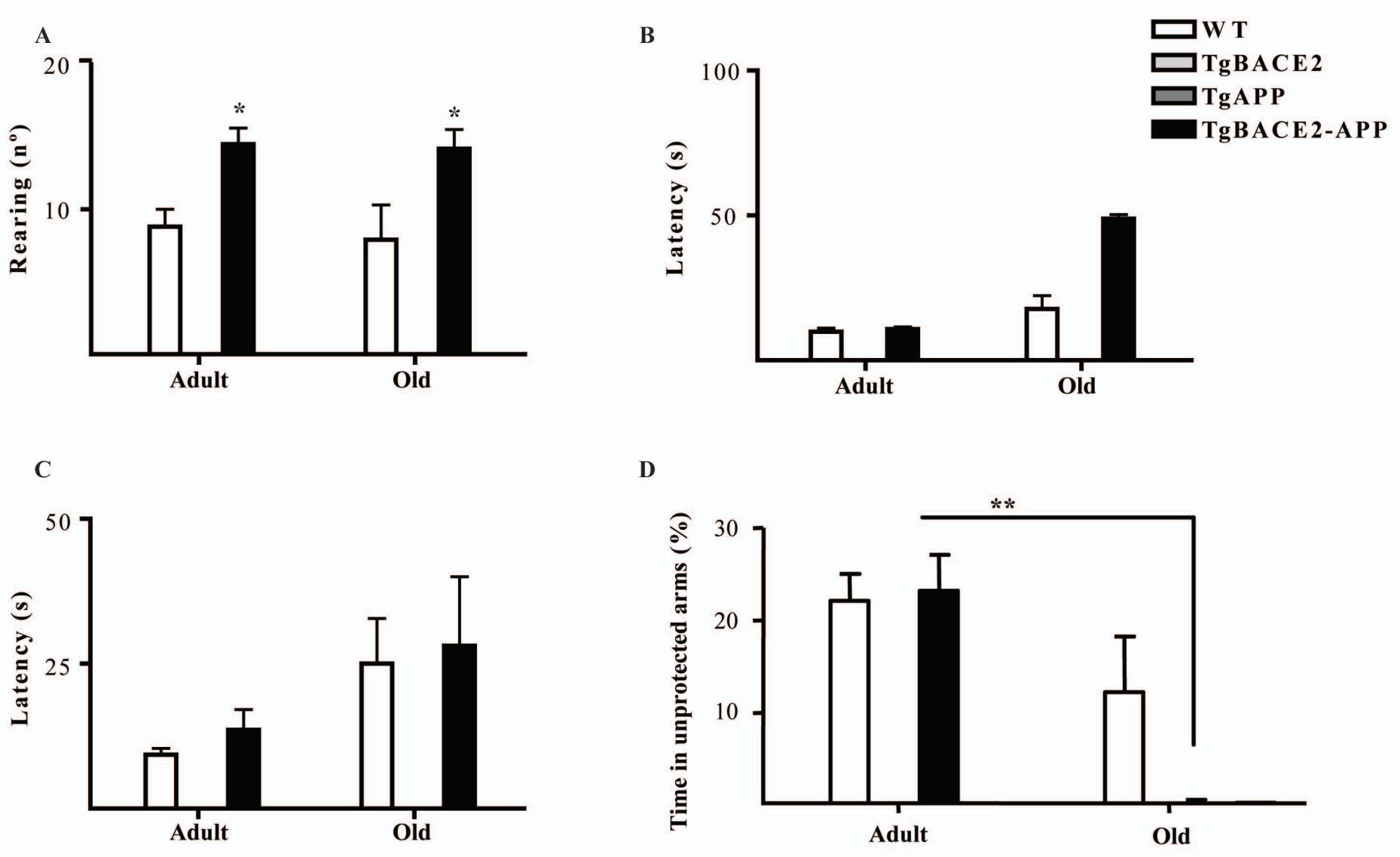
A
20
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Rearing (n°)
*
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Adult
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TgBACE2
TgAPP
TgBACE2-APP
100
50
Latency (s)
Adult
Old
C
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Latency (s)
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Old
D
**
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20
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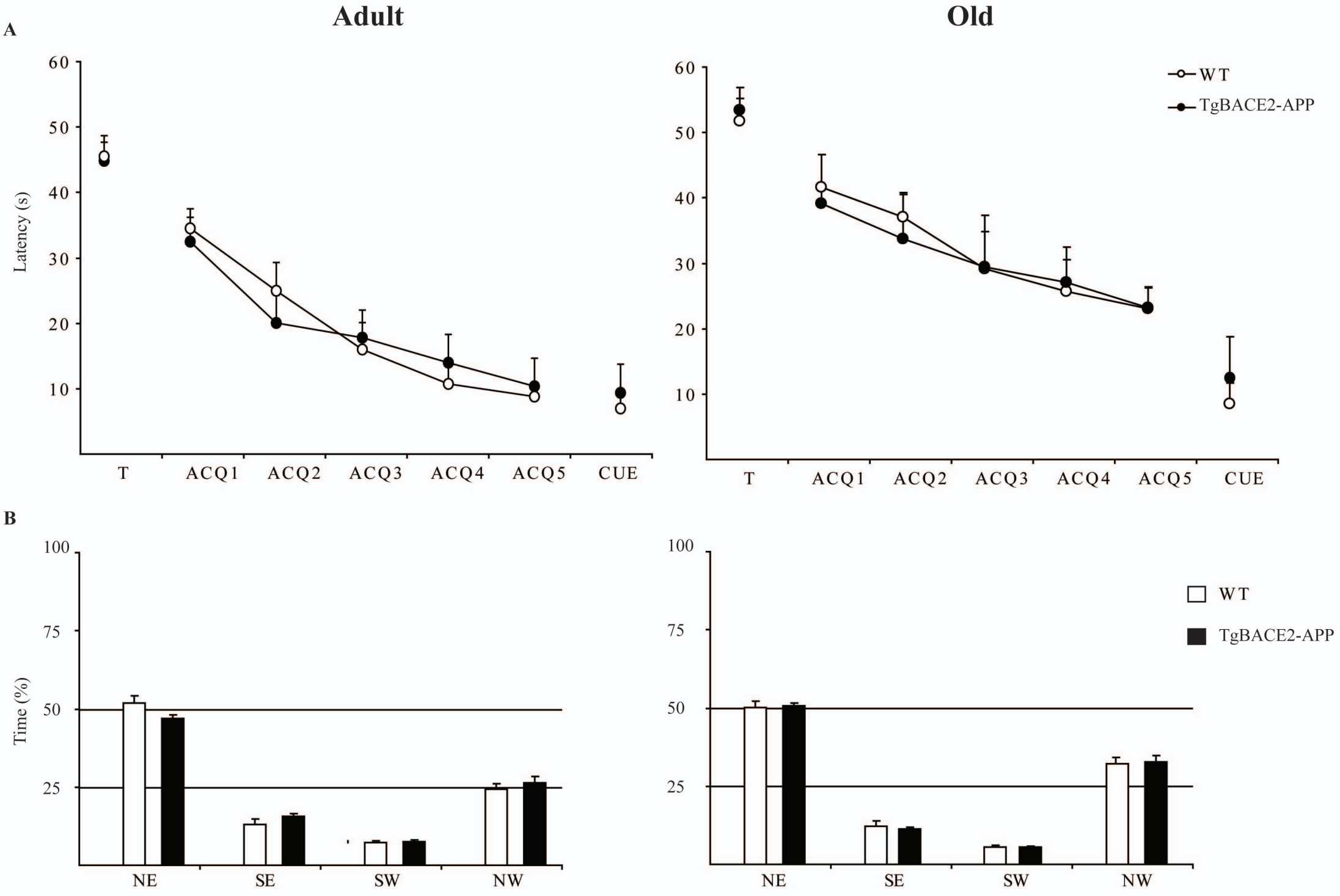
Time in unprotected arms (%)
Adult
Old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Old
Latency (s)
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10
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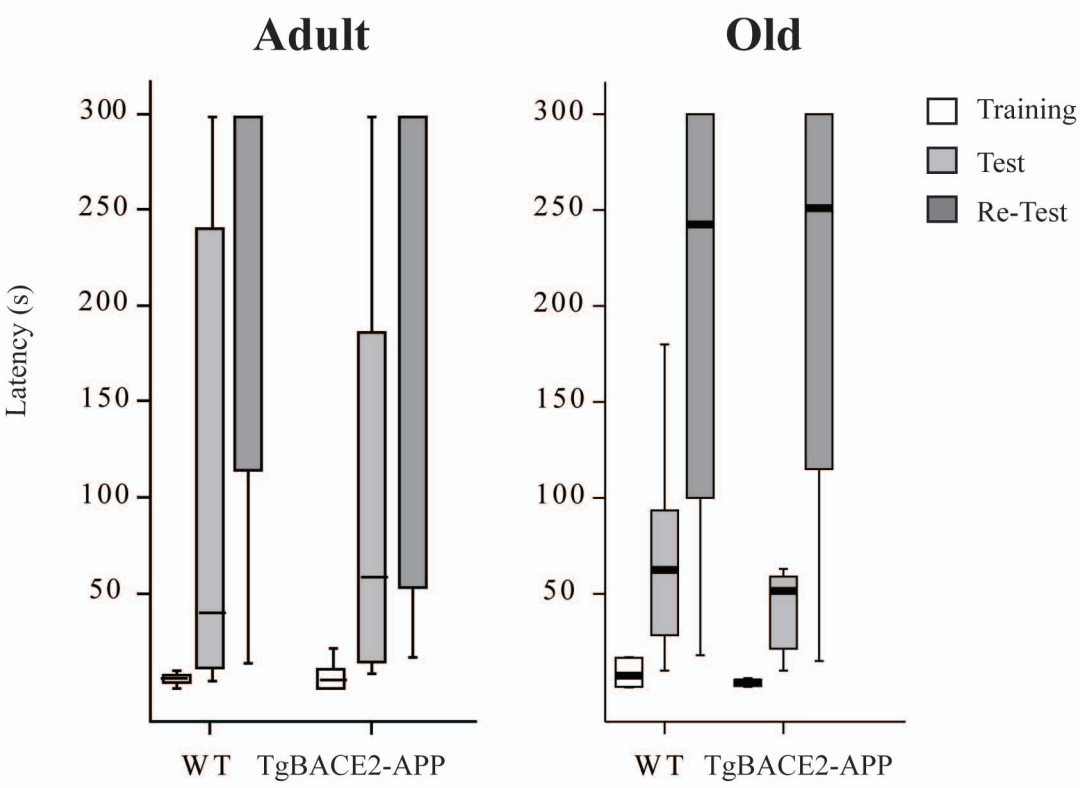
ACQ1
ACQ2
ACQ3
ACQ4
ACQ5
CUE
WT
TgBACE2-APP
B
Time (%)
100
75
50
25
NE
SE
SW
NW
WT
TgBACE2-APP


Adult
Old
Latency (s)
300
250
200
150
100
50
Training
Test
Re-Test
WT
TgBACE2-APP
WT
TgBACE2-APP

Supplementary Table 1. **SHIRPA and neuromotor tests**

			Adult		Old	
			Wild-type	**TgBACE2-APP**	**Wild-type**	**TgBACE2-APP**
Reflex	Righting Reflex	Landing onto 4 paws	100%	100%	100%	100%
	Corneal	Present	100%	100%	100%	100%
	Pinna	Present	100%	100%	100%	100%
	Toe pinch	Soft	0%	0%	0%	0%
		Mild	0%	0%	0%	0%
		Fast	0%	0%	30%	30%
		Vigorous	100%	100%	70%	70%
Response	Reaching response	Before contacting with the moustaches	100%	100%	40%	30%
		Initiating the rapprochement	0%	0%	60%	70%
	Startle	No response	0%	0%	0%	0%
		Backwards flick of the pinnae	100%	100%	100%	100%
		Startled response (1 cm)	0%	0%	0%	0%
	Touch escape	No reaction	30%	20%	0%	50%
		Response to strong touch	10%	20%	10%	20%
		Response to soft touch	60%	60%	80%	30%
		Flees prior to touch	0%	0%	10%	0%
Vision	Visual Placing	Extends legs before contacting the moustaches	100%	100%	100%	100%
Anxiety	Piloerection	Absent	100%	100%	100%	100%
	Tail elevation	No elevation	60%	60%	0%	10%
		Tail held out from the body	40%	40%	100%	90%
		Elevated (Straub tail)	0%	0%	0%	0%
Vestibular System	Positional passivity	Present	100%	100%	100%	100%
	Negative geotaxis	Turns and ascends	90%	80%	70%	60%
		Moves without turning	10%	20%	30%	40%
		No movement	0%	0%	0%	0%

		Adult		Old	
		Wild-type	**TgBACE2-APP**	**Wild-type**	**TgBACE2-APP**
Wire Suspension Test	Fell before 20s	0%	0%	0%	0%
	Remained in the centre	0%	0%	30%	10%
	Moved out of the centre	0%	0%	0%	10%
	Reached one of the ends	100%	100%	70%	80%
	Mean latency (s)	40	40	40	40
Coat Hanger Test	Prehensil reflex				
	Remained hanging for 5s	100%	100%	100%	100%
	Traction capacity				
	Did not lift-up the hind limbs	0%	0%	20%	60%
	Lift-up one hind limb	0%	0%	10%	10%
	Lift-up both hind limb	100%	100%	70%	30%
	Mean latency (s)	60	60	59,5	58,5
Muscle strength	Average (g)	60,2	68,5	51,9	55,5

There were no differences between genotypes. Data are expressed in percentage of mice attaining the indicated level, except for the latencies (seconds; s) and the muscle strength (grams; g).