



**HAL**  
open science

## One-year longitudinal evaluation of sensori-motor functions in APP751SL transgenic mice.

Camille Le Cudennec, Alexis Faure, M. Ly, Benoit Delatour

### ► To cite this version:

Camille Le Cudennec, Alexis Faure, M. Ly, Benoit Delatour. One-year longitudinal evaluation of sensori-motor functions in APP751SL transgenic mice.. *Genes, Brain and Behavior*, 2008, Suppl. 1, pp.83-91. 10.1111/j.1601-183X.2007.00374.x . hal-00320586

**HAL Id: hal-00320586**

**<https://hal.science/hal-00320586v1>**

Submitted on 25 May 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

C. Le Cudennec<sup>1,2</sup>  
A. Faure<sup>1</sup>  
M. Ly<sup>1</sup>  
B. Delatour<sup>1,\*</sup>

**One-year longitudinal evaluation of sensori-motor functions in APP<sub>751</sub>SL transgenic mice**

**Running title: Neurological impairments in APP mice.**

Date of submission: September, 05<sup>th</sup> 2007

Number of words in the Abstract: 241

Number of words in the Introduction: 596

Number of words in the Discussion: 1737

Keywords: Alzheimer's disease; sensorimotor functions; locomotor activity; aging; amyloid beta peptide; clinico-pathological correlations.

<sup>1</sup> Laboratoire de Neurobiologie de l'Apprentissage, de la Mémoire & de la Communication, NAMC, CNRS UMR 8620, Bât 446  
Université Paris-Sud  
91405 Orsay Cedex, FRANCE

<sup>2</sup> C. Le Cudennec is now at the Institut de Recherches Servier,  
Centre de Recherches de Croissy,  
Psychopharmacology Department,  
125, Chemin de Ronde,  
78290 - Croissy-sur-Seine, Paris, France.

\* Corresponding author

Benoît Delatour  
Laboratoire de Neurobiologie de l'Apprentissage, de la Mémoire & de la Communication,  
NAMC, CNRS UMR 8620, Bât 446  
Université Paris-Sud  
91405 Orsay Cedex, FRANCE  
Tel:33 (0)1 69 15 49 88  
Fax:33 (0)1 69 15 77 26  
Email [benoit.delatour@ibaic.u-psud.fr](mailto:benoit.delatour@ibaic.u-psud.fr)

## Abstract

Intracerebral amyloid-beta peptide (A $\beta$ ) deposition is considered to play a key role in Alzheimer's disease and is designated as a principal therapeutical target. The relationship between brain A $\beta$  levels and clinical deficits remains however unclear, both in human patients and in animal models of the disease.

The purpose of the present study was to investigate, in a transgenic mouse model of brain amyloidosis, the consequences of A $\beta$  deposition on basic neurological functions, using a longitudinal approach. Animals were phenotyped at different ages corresponding to graded neuropathological stages (from no extracellular A $\beta$  deposition to high amyloid loads). Sensory functions were evaluated by assessing visual and olfactory abilities and did not show any effects of the APP transgene. Motor functions were assessed using multiple experimental paradigms. Results showed that motor strength was considerably reduced in APP transgenic mice compared to control animals. No deficit was noted in a motor coordination test although APP transgenic mice displayed decreased locomotion on a stationary beam. Hypolocomotion was also observed in the standard open field test. Measures of anxiety obtained in the elevated plus-maze reveal some evidence of hyperanxiety in 15 months-old transgenic mice. Some of the neurological impairments showed by APP mice had an early onset and worsened with progressive aging, in parallel to gradual accumulation of A $\beta$  in brain parenchyma. Relationships between neuropathologically-assessed amyloid loads and behavioural deficits were further explored, and it was observed that motor strength deficits were correlated with cortical amyloid burden.

## Introduction

Alzheimer's disease (AD) has become a critical public health issue in our industrialized societies as the aging population continues to dramatically increase. From the pioneering studies of Alois Alzheimer (Alzheimer *et al.*, 1995), research has rapidly evolved and now relies on molecular and cellular biology approaches focusing on transgenic mice overexpressing mutated genes associated with familial AD (Delatour *et al.*, in press, German & Eisch, 2004, Higgins & Jacobsen, 2003, Van Dooren *et al.*, 2005). These murine models mainly replicate one hallmark neuropathological lesion of AD, which is parenchymal accumulation of amyloid-beta ( $A\beta$ ) peptides that subsequently form plaques. Such models derive from the so-called amyloid cascade hypothesis (Hardy & Higgins, 1992, Selkoe, 1991, Sommer, 2002) that was triggered by the pioneering work of Glenner, Wong, Beyreuther and Masters (Glenner & Wong, 1984, Masters & Beyreuther, 1989) and that posits accumulation of cerebral  $A\beta$  as a key primary event in the physiopathogeny of AD. Therapeutical assays at the moment largely rely upon the amyloid cascade hypothesis with the development of  $A\beta$ -lowering drugs being intensively explored.

Several fundamental questions and concerns remain to be addressed for a better understanding of AD's physiopathogeny and of the value/limits of animal models mimicking the disease process.

First of all, the behavioural phenotype of transgenic mice replicating AD neuropathology has been mainly studied using batteries of cognitive (learning and memory) tasks while non-cognitive aspects of behaviour have been less extensively investigated in these mice (see however recent studies: Adriani *et al.*, 2006, Arendash *et al.*, 2004, Lalonde *et al.*, 2005, Lalonde *et al.*, 2004, Leighty *et al.*, 2004, Pugh *et al.*, 2007, Vloeberghs *et al.*, 2006, Vloeberghs *et al.*, 2004, Wirths *et al.*, 2007). Refining screening of AD mutant mice to include (non-cognitive) neurological tests could therefore be of interest, especially in terms of face validity for the model. More importantly, longitudinal behavioural studies assessing repeated testing of the same mice at different ages have been rarely carried out and, most of the time, were restricted to cognitive evaluation (Chen *et al.*, 2000, McCool *et al.*, 2003, Savonenko *et al.*, 2003, Zhuo *et al.*, 2006). Such time-consuming studies are nevertheless of importance to finely evaluate the onset and evolution of behavioural impairments in relation with graded neuropathological stages.

The purpose of the present study was to investigate, in a transgenic mouse model bearing Swedish and London APP mutations and developing discrete cognitive impairments (Blanchard *et al.*, 2007, Blanchard *et al.*, 2003), the role of  $A\beta$  on (non-cognitive) neurological disorders. Primary screening (using a SHIRPA-like protocol) of this transgenic line was performed in the past and did not show any obvious effects of the mutated APP transgene in 9-months old transgenic mice, except lower body weights (Dr. O. Piot-Grosjean, personal communication). We therefore used a complementary behavioural battery involving more elaborated tests to evaluate neurological functions in these mutant mice. Animals were initially phenotyped at an early age preceding onset of extracellular  $A\beta$  accumulation (3 months-old). Sensory functions were evaluated by means of tests assessing visual (visual cliff test) and olfactory (buried food test) abilities. Motor functions were rated using three experimental paradigms: wire grid test, coat hanger test and stationary beam coordination test. Locomotion (and also some aspects of anxiety-related behaviours) were assessed with the standard open field test, and anxiety was evaluated in the elevated plus-maze task. Mice were then retested at 6 months of age (onset of brain amyloidosis) and finally at 15 months (high density of plaques) before being sacrificed to address clinico-pathological correlations between behavioural performances collected over a one-year period and histological/morphological markers developed by aged mice.

## **Material and methods**

### ***Experimental subjects***

The transgenic mice were generated and established according to standard procedures. Mice who had initially a hybrid genetic background (C57BL/6J [87.5%]×CBA [12.5%]), were subsequently backcrossed to C57BL/6J mice for six generations (for details, see Blanchard *et al.*, 2003). These transgenic mice overexpressed hAPP bearing Swedish (K670M/N671L) and London (V717I) mutations under the control of the neuron specific mouse Thy-1 promoter. The level of human APP overexpression was 5 to 7 time greater than endogenous murine APP levels in this transgenic line (Blanchard *et al.*, 2003).

The cohort used in the present study comprised 57 transgenic male mice (APP group) and 20 wild type non-transgenic male littermates used as controls (WT group). All mice were 6 weeks old at their arrival and behavioural testing was initiated at two months of age.

Male siblings were kept in the same cage (two to four mice per cage) under a 12-h light–dark cycle (light on: 7.00 a.m.) with food/water ad libitum and controlled humidity (50 %) and temperature (22°C) conditions.

All experiments were conducted in accordance with the ethical standards from French and European laws (European Communities Council Directive of 24 November 1986). The supervisor of the present study (B. Delatour) had received official agreements from the French Ministry of Agriculture to carry out research and experiments on animals (authorization No. 91-326). The general health of mice was regularly checked and body weights were assessed every week throughout the experimental period.

### ***Behavioural tests***

Neurological evaluation was carried out at 3, 6 and 15 months, corresponding to differentiated neuropathological stages in APP mice (3 months: no plaques; 6 months: onset of plaque deposition; 15 months: high amyloid load) as expected from known phenotype of the transgenic line used (Blanchard *et al.*, 2003). Neurological tests assessed sensorimotor functions, locomotor activity and anxiety-related behaviours.

Mice were moved to the experimental room 15 min. before each test.

### **Buried food test**

This test was adapted from the Jackson Laboratory phenotyping battery to assess basic olfactory functions. Animals were not food deprived prior to the test. A plastic cage (length, 40 cm; width, 16 cm; height, 24 cm) filled with wood chips was used. In one of the four corners of the cage, a small piece of cheese cracker was buried. Place of buried food was randomly changed between animals. Mice were placed in the center of the apparatus and their latency to find the cracker was recorded (cut off: 10 min). Wood chips were stirred between animals to homogenize odor gradients.

### **Visual cliff test**

Gross visual acuity was evaluated using the visual cliff test. This test measures depth perception in mice by assessing avoidance behaviour of a cliff (Fox, 1965). Testing was carried out in an open-topped box (length, 75 cm; width, 75 cm; height, 60 cm). The box was made from opaque PVC and equipped with an elevated clear Plexiglas floor (40 cm-height from the floor) defining a safe area (visually opaque floor) and an unsafe zone (above a cliff). An elevated rectangular platform (length, 30 cm; width, 7 cm; height, 5,5 cm) was placed at the frontier between safe and unsafe areas. Mice were deposited on the platform, therefore

avoiding immediate whiskers contacts with the floor, and latency and side of step down were recorded (cut off: 10 min).

### **Wire grid test**

Motor strength was measured with the wire grid test. Strength suspension (four paws) was assessed on a grid bordered with masking tape to prevent the mouse from walking off the edge. The mouse was placed on the center of the grid that was slowly turned upside down at a 20 cm-height above the floor. Latency to fall was recorded (cut off: 1 min).

### **Coat hanger test**

The coat hanger test was used to evaluate motor coordination. An iron horizontal bar (1.3 mm diameter) was placed at a height of 30 cm from the floor; a cardboard wall was inserted at each end of the bar in order to prevent mice from escaping sideways. Mice were placed in the middle part of the horizontal bar. Each trial began only when the forepaws gripped the bar at the moment of release. Three trials were recorded (intertrial interval: 5 min) and after each trial, mice were re-isolated. The latencies of the traction reflex (ie time the animal took to catch the bar by one of its hind paws) and alternatively the latencies before falling (cut off: 30 s), were measured.

### **Stationary beam test**

Motor coordination was also assessed using the stationary beam test. A round wooden beam (2.3 cm of diameter) was placed at a height of 35 cm from the floor and divided into seven 10-cm long segments. In order to avoid any escape, two cardboards flanked the beam. Mice were placed on the middle of the beam and number of segment crossed (4 paws criterion), latencies before falling, and number of falls were measured in a single 4-trials session. The cut-off was set to 1 min per trial and the intertrial interval to 10 min.

### **Open field test**

Locomotor activity was measured in a squared open field (50x50 cm; luminosity: 35 Lux) with black walls 30-cm high. The arena was divided into 25 squares (16 peripheral and 9 central). Each animal was placed in the center of the arena and allowed to freely explore for 10 min. The total number of square crossings (horizontal transitions) and rearings (vertical activity) were recorded for each animal. Collection of behavioural measures was performed using a laptop computer equipped with home-made software and connected to a numeric cam recorder.

### **Elevated plus-maze test**

Anxiety was evaluated in a cross-maze (length, 28 cm; width, 5 cm; height from floor, 40 cm; overall luminosity in open arms: 70 Lux) with two opposing open arms and two enclosed arms with three walls (height, 16 cm). Mice were placed in the central region of the maze and the number of entries and time spent in each arms were measured for a 5-min period using home-made software for behavioural analysis.

### **Histology**

After completion of longitudinal behavioural testing, mice were sacrificed with an overdose of sodium pentobarbital and were perfused transcardially with saline solution. After removal, the brains were weighed and photographed (ventral, dorsal and medial faces after separating the two hemispheres) using an Olympus C5050-Z camera fixed on a macro focusing stage. From macro-photographs, the brain length was measured at the midline level using the measure tool of Photoshop CS2 (Adobe, Paris, France) and converted to millimeters.

After one-week fixation in 10% formalin and subsequent cryoprotection in 20% glycerin and 2% dimethylsulfoxide in 0.1M phosphate buffer (PB) right hemispheres were sectioned (frontal 40µm-thick sections) on a freezing microtome. Twelve batches of serial sections (ranging from the frontal pole to the end of the caudal part of the hippocampus) were collected. Two series of free floating sections were immediately rinsed with 0.1 M PB and mounted on Superfrost+ glass slides before being processed for Nissl and amyloid staining. Remaining tissue was frozen in cryoprotectant as backup material. Nissl stain made use of thionin and labeling of amyloid deposits was done by standard Congo red staining (30 min in a 80% ethanol solution saturated with congo red and sodium chloride (adapted from Puchtler *et al.*, 1962)). Each slice was then digitized using a Super CoolScan 8000 ED scanner (Nikon, Champigny sur Marne, France) with a 4000 dpi in-plane digitization resolution (pixel size 6.35 µm<sup>2</sup>).

Amyloid loads were quantified on Congo red stained material using computer-based thresholding methods (see Figure 1a). Scans were prepared using Photoshop CS2 to outline whole brain sections or selected regions of interest (ROI). Images were then processed with ImageJ freeware (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2006) using a macrocommand that executed the following operations: (1) R, G, B color component adjustment, (2) global automated thresholding based on entropy criterion, and (3) morphometric filtering based on circularity and size constraints.

Regional amyloid loads were expressed as percent of tissue surface stained by the Congo red dye (Delatour *et al.*, 2006, El Tannir El Tayara *et al.*, 2006) that corresponds to the proportion of plaques volume according to Delesse principle (Delesse, 1847). Evaluation of amyloid loads were performed for the whole brain (cortical plus subcortical areas) and in different regions of interest: the cortical mantle, (i.e. isocortex plus hippocampus that are the ROIs displaying the highest amyloid burden), the motor cortex and finally the amygdala (lateral, basolateral and basomedial nuclei delimited by the external capsula). Quantitative analyses were made on several serial sections (whole brain: n= 15 sections/mouse; motor cortex: n= 6sections; amygdala: n= 4 sections). Such sampling, associated with the large number of processed mice, meets requirements defined to assess, with good precision, amyloid loads in brain tissue of transgenic mice (Fishman *et al.*, 2001).

### **Statistical analysis**

Almost all behavioural parameters were analyzed using two-factors ANOVA with repeated measures (with Genotype as the between-subjects factor and Age as the within-subject factor) using Statistica software (StatSoft, Inc., Tulsa, OK, USA). Differences between genotypes on brain weights and lengths were assessed by means of the Student t-test (Statistica Software). The visual cliff test's performances were analysed using non-parametric Mann-Whitney U and Cochran's Q tests (Statistica Software). Multivariate analysis of behavioural data was subsequently performed using SIMCA-P (Umetrics, Umea, Sweden). Normality of amyloid loads distribution was assessed using the Lilliefors test (Lilliefors, 1967). Correlative analysis between morphological and behavioural measures were based on the Pearson coefficient of correlation (Statistica Software) and Bonferroni and Holm corrections for multiple comparisons were subsequently performed to adjust p values.

### **Results**

APP mice showed enhanced lethality at young ages (before 4 months) with 23 out of 57 mice (40%) dying before the completion of the study for unknown reasons (no seizures detected, no relationship with body weight loss, no apparent alteration of the gross phenotype). Statistical analyses were only carried out using mice that completed the whole one-year training set.

## **Behaviour**

### **Sensory functions**

The latency to locate a fragrant cue (**buried food test**) was not significantly different between genotypes ( $F < 1$ ; see Table I), underlining similar basic olfactory functions in WT and APP mice. Performance did not vary across ages ( $F < 1$ ) and the interaction between Genotype and Age factors was not significant either ( $F(2,100) = 1.474$ ; ns).

When evaluated in the **visual cliff test**, both WT and APP mice demonstrated clear avoidance behaviour toward the unsafe (cliff) side of the apparatus (see Table I), with no differences between genotypes (Mann-Whitney test;  $U_s > 250$ ; ns). Visual detection of the cliff (unsafe) area was not affected by aging, neither in WT (Cochran test;  $Q = 1$ ; ns) nor in APP mice ( $Q = 0.316$ ; ns).

### **Motor functions**

Analysis of neurological performance in the **wire grid test** revealed a significant reduction of motor strength in APP mice as compared to WT mice (Genotype effect:  $F(1,50) = 11.552$ ;  $p < .0025$ ; see Table II). Genotype effect was confirmed at 3 and 15 months but not at 6 months of age. A global decline of performance was noted with progressive aging ( $F(2,100) = 13.694$ ;  $p < .001$ ), affecting both WT ( $F(2,34) = 3.995$ ;  $p < .05$ ) and APP mice ( $F(2,66) = 15.815$ ;  $p < .001$ ), with no significant interaction between Age and Genotype factors ( $F(2,100) = 2.553$ ;  $p > .082$ ). Mean latency of the traction reflex measured in the **coat hanger test** was not affected by Genotype ( $F(1,50) = 2.036$ ; see Table II) but did significantly fluctuate across ages ( $F(2,100) = 35.975$ ;  $p < .001$ ) in both genotypes. The interaction Genotype x Age was not significant ( $F(2,100) = 1.492$ ; ns). The latency to fall from the coat hanger was also measured as an alternative index of motor strength (Table II). Analysis of this variable indicated a decay of performance with aging ( $F(2,100) = 48.344$ ;  $p < .001$ ) as well as significant Genotype and Genotype x Age effects ( $F(1,50) = 9.976$ ;  $p < .005$  and  $F(2,100) = 3.483$ ;  $p < .05$  respectively). Subsequent analysis underlined that both WT and APP mice showed a drop in latency to fall with aging (WT:  $F(2,34) = 10.358$ ;  $p < .001$  and APP:  $F(2,66) = 55.214$ ;  $p < .001$ ). Group comparisons indicated no differences between genotypes at 3 and 6 months of age ( $F(1,50) = 1.216$ ; ns and  $F < 1$  respectively) but a significantly impaired performance of 15-months old APP mice as compared to age-matched WT mice ( $F(1,50) = 15.445$ ;  $p < .001$ ).

Motor coordination was evaluated with the **stationary beam test**. Analysis of the number of crossed segments revealed both a Genotype effect ( $F(1,50) = 6.857$ ;  $p < .025$ ) as well as Age and Age x Genotype effects ( $F_s(2,100) > 5.2$ ;  $p_s < .01$ ; Table II), with APP mice showing decreased activity (in comparison to WT mice) at 15 months of age ( $F(1,50) = 29.767$ ;  $p < .001$ ) but not at younger ages ( $F_s(1,50) < 1.749$ ; ns). Old APP mice also showed reduced latency to fall when compared to 15-months old WT mice ( $F_s(1,50) = 10.16$ ;  $p_s < .0025$ ; Table II).

### **Locomotion and anxiety-related behaviours**

Locomotor evaluation was carried out in the standard **open field test**. Analysis of horizontal activity (see Table III) showed an effect of the Genotype factor ( $F(1,39) = 31.966$ ;  $p < .001$ ) with significant Age and Genotype x Age effects ( $F_s(2,98) > 5.254$ ;  $p_s < .01$ ). APP mice demonstrated clear evidence of decreased locomotion at all tested ages ( $p_s < .005$ ) and worsening of performance with progressive aging was noted in this genotype ( $F(2,64) = 20.732$ ;  $p < .001$ ) but not in control WT mice ( $F(2,34) = 1.168$ ; ns). Vertical activity was also measured during testing and was found to noticeably decrease with aging ( $F(2,98) = 17.234$ ;  $p < .001$ ; see Table III), with no effect of Genotype ( $F(1,49) = 2.763$ ; ns) nor interaction between Genotype and Age factors ( $F(2,98) = 1.15$ ; ns).



As expected, in the **elevated plus-maze test**, mice spent more time in the closed arms than in the open arms ( $F(1,37)= 542.05$ ;  $p<.001$ ; see Table III) underlining avoidance of the most anxiogenic area of the apparatus. Analysis of variance showed no effects of Genotype and Age factors (respectively  $F(1,37)= 3.68$ ; ns and  $F(2,74)= 1.69$ ; ns) but a significant Age x Genotype interaction ( $F(2,74)= 8.21$ ;  $p<.001$ ). Subsequent analysis revealed no differences between genotypes at 3 and 6 months of age ( $F_s<1$ ) but a significant effect of the Genotype factor at 15-months of age ( $F(1,47)= 12.381$ ;  $p<.001$ ). At this age, APP transgenic mice showed increased time spent in closed arms when compared to WT mice ( $F(1,37)= 4.154$ ;  $p<.05$ ). However the time spent in the open arms did not differ between the two genotypes ( $F<1$ ). To assess, in a complementary way, differential exploration of open and closed arms, a ratio was computed (number of entries in open arms divided by total number of entries in open and closed arms; see table III). This ratio gradually decreased with aging ( $F(2,7)= 20.6$ ;  $p<.001$ ) with no effects of Genotype nor a significant interaction between Genotype and Age factors ( $F_s<1$ ).

### **Multivariate analysis of behavioural performances**

Partial least squares discriminant analysis was carried out on collected behavioural data with two major aims: (1) to find out, for each genotype, which dependent variables are the most affected by aging process; (2) to determine, at each time-point of the longitudinal study, which variables are the more efficient to discriminate between APP and WT phenotypes.

For control WT mice, the most discriminant variables between testing ages (Variable Influence on Projection - VIP>.08) were the vertical activity in the open field test and the latency to fall in the coat hanger test that both largely decreased with progressive aging. In the APP transgenic group, same analysis revealed as age-related discriminant parameters (VIP>0.8) a set of different variables: horizontal activity in the open field and stationary beam tests as well as latencies to fall in the coat hanger and stationary beam tests. All these variables were related to performance decline in aged APP mice.

At 3 and 6 months of age, multivariate analysis failed to correctly separate WT and APP neurological performances ( $Q^2_{cum}= .193$  and  $.397$  respectively). However older mice (15 months) were clearly differentiated by the Genotype factor ( $Q^2_{cum}= .652$ ). At this age, the most discriminant variables (VIP>.08) were the locomotor activity (open field and stationary beam tests) and the latencies to fall in different tests (coat hanger, wire grid and stationary beam tasks).

### **Morphological analysis**

#### **Gross morphology**

During the one-year period of behavioural testing, APP mice showed a constant lower body weight (10-20%) in comparison to control non-transgenic mice (see table IV). Analysis of variance carried out on mean body weights obtained at 3, 6 and 15-months of age confirmed a Genotype effect ( $F(1,50)= 111.02$ ;  $p<.001$ ). Notably no correlations were noted between body weights and neurological performances (data not shown).

Fresh brains were weighed and measured (see table IV). Brain weights were not different between genotypes ( $t(50)= 0.879$ ; ns) but APP mice showed anomalous increased brain length when compared to WT mice ( $t(50)= 3.179$ ;  $p<.005$ ).

#### **Neuropathology and clinico-pathological correlations**

Amyloid loads estimated from Congo red-stained slices ranged between 0.9% to 2.2% (whole brain) and between 0.95% and 5.37% (selected ROIs) of total sampled volumes (see Table IV and Figure 1 b). The amyloid loads showed normal distributions as assessed by Lilliefors tests.

No association was found between macroscopic observations (brain weights and lengths) in APP transgenic mice and amyloid loads even though a negative trend was noted between brain weight and total brain amyloid load ( $r = -0.32$ ;  $p = 0.062$ ).

Correlation analyses were performed to study the impact of neuropathological markers (amyloid burden) upon neurological functions (only behavioural dependent variables associated with functional impairments in APP transgenic mice were selected for analysis:  $n = 6$ ). Performances at young ages were not correlated with (and therefore did not predict) amyloid loads evaluated at 15 months of age (data not shown). However, in old APP mice some associations were found between neuropathological staging and neurological performance obtained just before sacrifice. Measures of motor strength (latencies to fall) were hence negatively correlated with cortical amyloid loads in three different test (wire grid:  $r = -0.37$ ,  $p < .05$ ; coat hanger:  $r = -0.49$ ,  $p < .005$ ; stationary beam:  $r = -0.4$ ,  $p < .025$ ). Following p-values adjustments for multiple comparisons, a negative correlation between cortical amyloid load and motor strength was still observed, but only in the coat-hanger test ( $p < .025$ ). In a similar way, amyloid load in the sole motor cortex was correlated with motor strength measure (coat hanger:  $r = -0.49$ ,  $p < .005$ ) and the correlation was preserved after adjustments for multiple comparisons ( $p < .025$ ). Subsequent analysis showed that mice with “low” cortical amyloid loads presented better performance than mice with “high” amyloid burden (Figure 2). By contrast, no associations were found between locomotor activity measures (open field and stationary beam tests) and amyloid burden in the cortex and in the whole brain ( $r_s < 0.25$ ; ns). Additionally, no significant correlations were observed between amyloid load in the amygdala and anxiety related measures ( $r_s < .07$ ).

## Discussion

The paucity of behavioural longitudinal studies of transgenic mice modelling AD's neuropathology led us to carry out a one-year long analysis of mutated APP transgenic mice by phenotyping them in a battery of non-cognitive neurological tests. In a very recent study, Pugh and collaborators (Pugh *et al.*, 2007) performed, as we did, a longitudinal evaluation of neurological functions in AD transgenic mice (TASTPM line carrying both APP and presenilin 1 mutations); however no histological characterization of the studied mice was performed with a clinico-pathological aim. In the present study, neuropathological examination was therefore achieved after completion of behavioural testing to address correlations between plaque burden and neurological performances.

### **Summary of results**

Behavioural testing allowed us to evaluate the gross integrity of two sensory modalities that are essential for rodents (olfaction, vision) and did not reveal obvious deleterious effect of the mutated APP transgene on these functions.

On the contrary, motor strength was severely affected in APP mice that showed decreased latencies to fall in different tasks involving grip abilities (wire grid, coat hanger and stationary beam tests). Locomotor evaluation in the open field test revealed a hypoactivity of APP mice that worsened with progressive aging. Similarly, APP mice showed decreased locomotion in the stationary beam test; this deficit, however, was only observed in old (15 months) animals. It remains difficult to determine if this latter impairment was due to a motor coordination defect or to a lack of motor strength as APP mice concurrently showed normal traction reflex in the coat hanger test that also requires some motor coordination. Finally, anxiety-related behaviours did not differ between genotypes until the age of 15 months when APP mice exhibited some evidence of hyperanxiety (increased time spent in closed arms).

Multivariate between-groups analysis allowed to clearly dissociate the two genotypes at 15 months of age, on the basis of selected neurological measures (locomotor activity and motor strength/coordination performance). However, at younger ages (2 and 6 months), differences between APP and control WT mice were not statistically confirmed by discriminant analysis. Finally, neuropathological studies showed a relation between amounts of plaque deposition in the cortex and motor strength deficits. No other clinico-pathological correlations were found; in particular, the severe hypoactivity of APP mice was not associated with cortical amyloid load.

### **Neurological impairments in AD transgenic mice: relevance to human pathology**

Neurological symptoms, including motor impairments, are described during the course of AD (Franssen *et al.*, 1991, Goldman *et al.*, 1999, Mega *et al.*, 1996, Pettersson *et al.*, 2002) but are not emphasized in the general clinical picture of diseased patients. The etiopathogeny of these non-cognitive traits remains obscure and have been attributed to comorbidity such as parkinsonism or lewy bodies dementia (Hansen *et al.*, 1990, Leverenz & Sumi, 1986) or alternatively to white matter lesions (Xie *et al.*, 2006). As described in the present study, APP transgenic mice modelling AD neuropathology show neurological dysfunction that might be reminiscent of symptoms depicted in patients. We do believe however that those deficits in transgenic mice are qualitatively and quantitatively different than those described in human pathology.

For instance, one of the main clinical feature we observed in APP transgenics was a severe loss of motor strength. Such deficits, to our knowledge, have not been described in AD

patients. Motor weakness has been isolated as a prognostic indicator for survival in demented patients (Koutsavlis & Wolfson, 2000), but strength loss can not be considered as a common symptom in AD. Data gained from the animal literature are also in conflict. Mice with pathological accelerated senescence have deficits in a wire suspension test (Markowska *et al.*, 1998) and recently-engineered murine transgenic lines (bearing APP mutations plus knock-in mutations of the endogenous presenilin 1 gene) also show impairments in suspension tests (Wirhns *et al.*, 2007, Wirhns *et al.*, 2006). On the contrary, other transgenic lines (eg APP23, APPxPS1 $\Delta$ E9 models) do appear to have normal grip strength (Lalonde *et al.*, 2005, Lalonde *et al.*, 2004). These discrepancies might be related to variations in genetic constructions of the different lines (mutations and promoters used, genetic backgrounds of the strains) as well as to heterogeneity of the tested populations (in terms of age, sex etc.) that does not allow strict comparisons between studies.

A second major characteristic of APP transgenic mice in the present study was a decreased locomotor activity. While a number of studies paradoxically showed hyperactivity in APP transgenic mice (eg King & Arendash, 2002, King *et al.*, 1999, Lalonde *et al.*, 2003), patterns of hypoactivity similar to our observations have been reported in concurrent transgenic lines (Pugh *et al.*, 2007). Impairments in locomotor behaviour are also described during the course of AD, presumably in relation with disrupted circadian rhythms (Satlin *et al.*, 1991). However, human studies mostly outline abnormal hyperactivity traits in AD patients, which contrasts with our data obtained in transgenic mice that show, on the contrary, decreased locomotion. In the present study, old APP mice displayed some evidence of abnormal anxiety-related behaviours (increased time spent in closed arms of the elevated plus-maze). Although not consistently reported, similar observations of hyperanxiety have been described in other transgenic lines (Lee *et al.*, 2004, Savonenko *et al.*, 2003). Neuropsychiatric symptoms, including increased anxiety manifestations, are commonly depicted in AD patients (Cummings, 1997) and might hence be paralleled by results from transgenic mice studies. Finally, we did not find evidence either of reflex motor coordination deficit (coat hanger test) or of visual or olfactory impairments in APP transgenic mice. The latter results suggest that sensory functions are intact in those animals. Such conclusions should be nonetheless tempered by reports showing deficits of APP transgenics when trained to locate a visual cue (Hsiao *et al.*, 1996, King *et al.*, 1999), as such impairments might indicate a loss of visual acuity. Furthermore, it is known from human studies that basic sensory deficits (especially in the olfactory modality) can to some extent be compromised in AD patients (Doty, 1994).

### **Neurological impairments in AD transgenic mice: neuropathological correlates**

In light of the “amyloid cascade” hypothesis we explored the relationship between onset and magnitude of neurological deficits and amyloid burden in APP transgenic mice. Some of the depicted behavioural impairments were not observed in young amyloid-free animals and appeared later in mice with full blown pathology (see results obtained in the coat hanger, stationary beam and plus-maze tasks; see also results from multivariate analysis). Other deficits (i.e. decreased locomotion in the open field) were present at young ages but worsened with progressive aging in association with development of cerebral amyloidosis. More importantly, correlations between total cortex or motor cortex amyloid loads and neurological performances were obtained in selected tests assessing motor strength. Previous studies carried out in different transgenic lines also reported relationships between amyloid burden and defects in learning and memory tasks (Arendash *et al.*, 2001, Chen *et al.*, 2000, Chishti *et al.*, 2001, Gordon *et al.*, 2001, Hsiao *et al.*, 1996). To our knowledge, correlations between basic (non-cognitive) neurological impairments and levels of fibrillar insoluble A $\beta$  (aggregated Congo red-positive plaques) have not been previously reported (see however Leighty *et al.*,

2004). Importantly, it should be kept in mind that A $\beta$  deposition in the brain is an age-dependent process, might be associated with concurrent neuropathological alterations, and therefore could act as a confounding variable with no direct impact on behavioural dysfunction.

Furthermore, we did not find, systematic relationships between neurological deficits and amyloid loads: the severe hypoactivity of APP transgenics did not appear to be linked to plaques burden; also amyloid load in the amygdala was not correlated to measures of anxiety in transgenic mice. It should be noted that several studies have claimed a lack of a direct association between A $\beta$  levels and behavioural decline (Holcomb *et al.*, 1998, Howlett *et al.*, 2004, Trinchese *et al.*, 2004), especially those showing no deficits in mice with high amyloid loads (Bizon *et al.*, 2006, Blanchard *et al.*, 2007). More importantly, a number of neurological deficits illustrated in the present study had a very early onset (locomotor hypoactivity, loss of motor strength in the wire grid test), prior to the appearance of A $\beta$  plaques. These observations, already reported in the literature and showing behavioural impairments in transgenic mice in the absence of fibrillar A $\beta$  deposition (Holcomb *et al.*, 1998, Lee *et al.*, 2004, Richardson *et al.*, 2003, Van Dam *et al.*, 2003), shed supplementary doubt on the causal and direct role of amyloid deposition on neurological and cognitive disruptions. Recent evidence from the literature has indicated that small A $\beta$  peptides assemblies that pre-exist to protofibrillar and aggregated deposits might have neurotoxic and synaptotoxic effects as well as deleterious action on cognitive functions (for recent studies, see Cheng *et al.*, 2007, Lesne *et al.*, 2006; for reviews, see Rowan *et al.*, 2005, Walsh & Selkoe, 2007). A $\beta$  oligomers, that were not analyzed in the present study, might therefore have a causal role in the onset and evolution of neurological deficits we described in APP transgenic mice. This point remains to be addressed in the future

Finally, various brain alterations directly or indirectly linked to A $\beta$  accumulation might concurrently be responsible for the neurological phenotype we described in APP transgenic mice. First, A $\beta$  can lead to disturbed neuronal trafficking and peripheral axonopathy. Such lesions have been associated to deficiencies in motor performances (Wirhth *et al.*, 2007, Wirhth *et al.*, 2006). Second, brain atrophies have been depicted in different APP transgenic lines (Delatour *et al.*, 2006, Gonzalez-Lima *et al.*, 2001, Valla *et al.*, 2006) and include severe callosal agenesis and reduced midbrain and internal capsule volumes. It is reasonable to point to these anomalies, presumably due to a neurodevelopmental defect, as impeding brain functioning and to have a clinical translation in terms of neurological symptoms.

In summary, we found evidence of neurological impairments in transgenic mice carrying human APP mutations and developing amyloid plaques from the age of 6-months. These observations have to be added to large datasets from the literature showing cognitive alterations in similar transgenic lines, especially to avoid misinterpretations as low performances in higher functions tests can simply result from decreased sensorimotor abilities. Major neurological deficits in APP transgenics concerned loss of motor strength and a decrease locomotor behaviour. These impairments are only partially similar to those found in AD patients and might be a characteristic of mice models. We found some support to the assumption that A $\beta$  accumulation was a key factor to the observed deficits but alternative hypotheses remain to be explored.

## Figure and table legends

### **Table I**

Performance in tests of sensory functions, in wild-type (WT) and APP transgenic (APP) mice. For each genotype performance is expressed as mean  $\pm$  SEM. \* denotes a genotype effect ( $p < .05$ ).

### **Table II**

Motor performance in wild-type (WT) and APP transgenic (APP) mice. For each genotype performance is expressed as mean  $\pm$  SEM. \* denotes a genotype effect ( $p < .05$ ).

### **Table III**

Measures of locomotor and anxiety-related behaviours in wild-type (WT) and APP transgenic (APP) mice.

The NbO/Nb(O+C) ratio represents the number of entries in open (O) arms divided by total number of entries in open and closed (C) arms

For each genotype performance is expressed as mean  $\pm$  SEM. \* denotes a genotype effect ( $p < .05$ ).

### **Table IV**

Summary of morphological measures in wild-type (WT) and APP transgenic (APP) mice.

For each genotype performance is expressed as mean  $\pm$  SEM. \* denotes a genotype effect ( $p < .05$ ).

### **Figure 1**

Neuropathological findings in APP transgenic mice.

(a). Illustration of automated processing to estimate amyloid loads (left : raw image; right: thresholded image with outlines of detected plaques). (b). Distribution of individual amyloid loads (% tissue stained by the Congo red dye) in the whole brain and in the cortex (horizontal bars represent groups means).

### **Figure 2**

Relation between brain amyloidosis and motor strength.

The population of APP transgenic mice was subdivided in two sub-populations with low (LOAD-low) or high (LOAD-high) cortical amyloid burden. These sub-populations were constituted apart the group median value of cortical amyloid load ( $>$  or  $<$  to 2.7 %).

Motor strength was evaluated by measuring latencies to fall in three different tasks (CH: coat hanger; WG: wire grid; SB: stationary beam). In each tests, latency to fall was reduced in mice with the highest loads ( $p < .05$ ).

## Bibliography

- Adriani, W., Ognibene, E., Heuland, E., Ghirardi, O., Caprioli, A. & Laviola, G. (2006) Motor impulsivity in APP-SWE mice: a model of Alzheimer's disease. *Behav Pharmacol*, **17**, 525-533.
- Alzheimer, A., Stelzmann, R.A., Schnitzlein, H.N. & Murtagh, F.R. (1995) An English translation of Alzheimer's 1907 paper, "Über eine eigenartige Erkrankung der Hirnrinde". *Clin Anat*, **8**, 429-431.
- Arendash, G.W., King, D.L., Gordon, M.N., Morgan, D., Hatcher, J.M., Hope, C.E. & Diamond, D.M. (2001) Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. *Brain Res*, **891**, 42-53.
- Arendash, G.W., Lewis, J., Leighty, R.E., McGowan, E., Cracchiolo, J.R., Hutton, M. & Garcia, M.F. (2004) Multi-metric behavioral comparison of APP<sup>sw</sup> and P301L models for Alzheimer's disease: linkage of poorer cognitive performance to tau pathology in forebrain. *Brain Res*, **1012**, 29-41.
- Bizon, J., Prescott, S. & Nicolle, M.M. (2006) Intact spatial learning in adult Tg2576 mice. *Neurobiol Aging*, **28**, 440-6
- Blanchard, J., Martel, G., Guillou, J.L., Nogues, X. & Micheau, J. (2007) Impairment of spatial memory consolidation in APP(751SL) mice results in cue-guided response. *Neurobiol Aging*, **Mar 9**, [Epub ahead of print].
- Blanchard, V., Moussaoui, S., Czech, C., Touchet, N., Bonici, B., Planche, M., Canton, T., Jedidi, I., Gohin, M., Wirths, O., Bayer, T.A., Langui, D., Duyckaerts, C., Tremp, G. & Pradier, L. (2003) Time sequence of maturation of dystrophic neurites associated with Abeta deposits in APP/PS1 transgenic mice. *Exp Neurol*, **184**, 247-263.
- Chen, G., Chen, K.S., Knox, J., Inglis, J., Bernard, A., Martin, S.J., Justice, A., McConlogue, L., Games, D., Freedman, S.B. & Morris, R.G. (2000) A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature*, **408**, 975-979.
- Cheng, I.H., Scarce-Levie, K., Legleiter, J., Palop, J.J., Gerstein, H., Bien-Ly, N., Puolivali, J., Lesne, S., Ashe, K.H., Muchowski, P.J. & Mucke, L. (2007) Accelerating amyloid-beta fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. *J. Biol. Chem.*, **282**, 23818-23828.
- Chishti, M.A., Yang, D.S., Janus, C., Phinney, A.L., Horne, P., Pearson, J., Strome, R., Zuker, N., Loukides, J., French, J., Turner, S., Lozza, G., Grilli, M., Kunicki, S., Morissette, C., Paquette, J., Gervais, F., Bergeron, C., Fraser, P.E., Carlson, G.A., George-Hyslop, P.S. & Westaway, D. (2001) Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J Biol Chem*, **276**, 21562-21570.
- Cummings, J.L. (1997) The Neuropsychiatric Inventory: assessing psychopathology in dementia patients. *Neurology*, **48**, S10-16.
- Delatour, B., Guegan, M., Volk, A. & Dhenain, M. (2006) In vivo MRI and histological evaluation of brain atrophy in APP/PS1 transgenic mice. *Neurobiol Aging*, **6**, 835-847.
- Delatour, B., Le Cudennec, C., El Tannir El Tayara, N. & Dhenain, M. (in press) Transgenic models of Alzheimer's pathology: success and caveats. In Welsh, E.M. (eds), *Progress in Alzheimer's Disease Research*. Nova Science Publishers.
- Delesse, M. (1847) Procédé mécanique pour déterminer la composition des roches. *C R Acad Sci*, **25**, 544-545.

- Doty, R.L. (1994) Olfactory dysfunction in the elderly and in Alzheimer's disease. In Kurihara, K., Suzuki, N. & Ogawa, H. (eds), *Olfaction and Taste XI*. Springer-Verlag, Berlin, pp. 597-601.
- El Tannir El Tayara, N., Delatour, B., Le Cudennec, C., Guegan, M., Volk, A. & Dhenain, M. (2006) Age-related evolution of amyloid burden, iron load, and MR relaxation times in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis*, **22**, 199-208.
- Fishman, C.E., Cummins, D.J., Bales, K.R., DeLong, C.A., Esterman, M.A., Hanson, J.C., White, S.L., Paul, S.M. & Jordan, W.H. (2001) Statistical aspects of quantitative image analysis of beta-amyloid in the APP(V717F) transgenic mouse model of Alzheimer's disease. *J Neurosci Methods*, **108**, 145-152.
- Fox, M.W. (1965) The visual cliff test for the study depth perception in the mouse. *Anim Behav*, **13**, 232-233.
- Franssen, E.H., Reisberg, B., Kluger, A., Sinaiko, E. & Boja, C. (1991) Cognition-independent neurologic symptoms in normal aging and probable Alzheimer's disease. *Arch Neurol*, **48**, 148-154.
- German, D.C. & Eisch, A.J. (2004) Mouse models of Alzheimer's disease: insight into treatment. *Rev Neurosci*, **15**, 353-369.
- Glenner, G.G. & Wong, C.W. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun*, **120**, 885-890.
- Goldman, W.P., Baty, J.D., Buckles, V.D., Sahrman, S. & Morris, J.C. (1999) Motor dysfunction in mildly demented AD individuals without extrapyramidal signs. *Neurology*, **53**, 956-962.
- Gonzalez-Lima, F., Berndt, J.D., Valla, J.E., Games, D. & Reiman, E.M. (2001) Reduced corpus callosum, fornix and hippocampus in PDAPP transgenic mouse model of Alzheimer's disease. *Neuroreport*, **12**, 2375-2379.
- Gordon, M.N., King, D.L., Diamond, D.M., Jantzen, P.T., Boyett, K.V., Hope, C.E., Hatcher, J.M., DiCarlo, G., Gottschall, W.P., Morgan, D. & Arendash, G.W. (2001) Correlation between cognitive deficits and Abeta deposits in transgenic APP+PS1 mice. *Neurobiol Aging*, **22**, 377-385.
- Hansen, L., Salmon, D., Galasko, D., Masliah, E., Katzman, R., DeTeresa, R., Thal, L., Pay, M.M., Hofstetter, R., Klauber, M. & et al. (1990) The Lewy body variant of Alzheimer's disease: a clinical and pathologic entity. *Neurology*, **40**, 1-8.
- Hardy, J.A. & Higgins, G.A. (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science*, **256**, 184-185.
- Higgins, G.A. & Jacobsen, H. (2003) Transgenic mouse models of Alzheimer's disease: phenotype and application. *Behav Pharmacol*, **14**, 419-438.
- Holcomb, L., Gordon, M.N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C.M., Eckman, C., Younkin, S., Hsiao, K. & Duff, K. (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med*, **4**, 97-100.
- Howlett, D.R., Richardson, J.C., Austin, A., Parsons, A.A., Bate, S.T., Davies, D.C. & Gonzalez, M.I. (2004) Cognitive correlates of Abeta deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. *Brain Res*, **1017**, 130-136.
- Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. & Cole, G. (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science*, **274**, 99-102.
- King, D.L. & Arendash, G.W. (2002) Behavioral characterization of the Tg2576 transgenic model of Alzheimer's disease through 19 months. *Physiol Behav*, **75**, 627-642.



- King, D.L., Arendash, G.W., Crawford, F., Sterk, T., Menendez, J. & Mullan, M.J. (1999) Progressive and gender-dependent cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. *Behav Brain Res*, **103**, 145-162.
- Koutsavlis, A.T. & Wolfson, C. (2000) Elements of mobility as predictors of survival in elderly patients with dementia: findings from the Canadian Study of Health and Aging. *Chronic Dis Can*, **21**, 93-103.
- Lalonde, R., Dumont, M., Staufenbiel, M. & Strazielle, C. (2005) Neurobehavioral characterization of APP23 transgenic mice with the SHIRPA primary screen. *Behav Brain Res*, **157**, 91-98.
- Lalonde, R., Kim, H.D. & Fukuchi, K. (2004) Exploratory activity, anxiety, and motor coordination in bigenic APP<sup>swe</sup> + PS1/DeltaE9 mice. *Neurosci Lett*, **369**, 156-161.
- Lalonde, R., Lewis, T.L., Strazielle, C., Kim, H. & Fukuchi, K. (2003) Transgenic mice expressing the betaAPP(695)SWE mutation: effects on exploratory activity, anxiety, and motor coordination. *Brain Res*, **977**, 38-45.
- Lee, K.W., Lee, S.H., Kim, H., Song, J.S., Yang, S.D., Paik, S.G. & Han, P.L. (2004) Progressive cognitive impairment and anxiety induction in the absence of plaque deposition in C57BL/6 inbred mice expressing transgenic amyloid precursor protein. *J Neurosci Res*, **76**, 572-580.
- Leighty, R.E., Nilsson, L.N., Potter, H., Costa, D.A., Low, M.A., Bales, K.R., Paul, S.M. & Arendash, G.W. (2004) Use of multivariate statistical analysis to characterize and discriminate between the performance of four Alzheimer's transgenic mouse lines differing in A $\beta$  deposition. *Behav Brain Res*, **153**, 107-121.
- Lesne, S., Koh, M.T., Kotilinek, L., Kaye, R., Glabe, C.G., Yang, A., Gallagher, M. & Ashe, K.H. (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature*, **440**, 352-357.
- Leverenz, J. & Sumi, S.M. (1986) Parkinson's disease in patients with Alzheimer's disease. *Arch Neurol*, **43**, 662-664.
- Lilliefors, H. (1967) On the Kolmogorov-Smirnov test for normality with mean and variance unknown. *Journal of the American Statistical Association*, **62**, 399-402.
- Markowska, A.L., Spangler, E.L. & Ingram, D.K. (1998) Behavioral assessment of the senescence-accelerated mouse (SAM P8 and R1). *Physiol Behav*, **64**, 15-26.
- Masters, C.L. & Beyreuther, K.T. (1989) The pathology of the amyloid A4 precursor of Alzheimer's disease. *Ann Med*, **21**, 89-90.
- McCool, M.F., Varty, G.B., Del Vecchio, R.A., Kazdoba, T.M., Parker, E.M., Hunter, J.C. & Hyde, L.A. (2003) Increased auditory startle response and reduced prepulse inhibition of startle in transgenic mice expressing a double mutant form of amyloid precursor protein. *Brain Res*, **994**, 99-106.
- Mega, M.S., Cummings, J.L., Fiorello, T. & Gornbein, J. (1996) The spectrum of behavioral changes in Alzheimer's disease. *Neurology*, **46**, 130-135.
- Pettersson, A.F., Engardt, M. & Wahlund, L.O. (2002) Activity level and balance in subjects with mild Alzheimer's disease. *Dement Geriatr Cogn Disord*, **13**, 213-216.
- Puchtler, H., Sweat, F. & Levine, M. (1962) On the binding of Congo red by amyloid. *J Histochem Cytochem*, **10**, 355-364.
- Pugh, P.L., Richardson, J.C., Bate, S.T., Upton, N. & Sunter, D. (2007) Non-cognitive behaviours in an APP/PS1 transgenic model of Alzheimer's disease. *Behav Brain Res*, **178**, 18-28.
- Richardson, J.C., Kendal, C.E., Anderson, R., Priest, F., Gower, E., Soden, P., Gray, R., Topps, S., Howlett, D.R., Lavender, D., Clarke, N.J., Barnes, J.C., Haworth, R., Stewart, M.G. & Rupniak, H.T. (2003) Ultrastructural and behavioural changes precede amyloid deposition in a transgenic model of Alzheimer's disease. *Neuroscience*, **122**, 213-228.

- Rowan, M.J., Klyubin, I., Wang, Q. & Anwyl, R. (2005) Synaptic plasticity disruption by amyloid beta protein: modulation by potential Alzheimer's disease modifying therapies. *Biochem Soc Trans*, **33**, 563-567.
- Satlin, A., Teicher, M.H., Lieberman, H.R., Baldessarini, R.J., Volicer, L. & Rheaume, Y. (1991) Circadian locomotor activity rhythms in Alzheimer's disease. *Neuropsychopharmacology*, **5**, 115-126.
- Savonenko, A.V., Xu, G.M., Price, D.L., Borchelt, D.R. & Markowska, A.L. (2003) Normal cognitive behavior in two distinct congenic lines of transgenic mice hyperexpressing mutant APP(SWE). *Neurobiol Dis*, **12**, 194-211.
- Selkoe, D.J. (1991) The molecular pathology of Alzheimer's disease. *Neuron*, **6**, 487-498.
- Sommer, B. (2002) Alzheimer's disease and the amyloid cascade hypothesis: ten years on. *Curr Opin Pharmacol*, **2**, 87-92.
- Trinchese, F., Liu, S., Battaglia, F., Walter, S., Mathews, P.M. & Arancio, O. (2004) Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice. *Ann Neurol*, **55**, 801-814.
- Valla, J., Schneider, L.E., Gonzalez-Lima, F. & Reiman, E.M. (2006) Nonprogressive transgene-related callosal and hippocampal changes in PDAPP mice. *Neuroreport*, **17**, 829-832.
- Van Dam, D., D'Hooge, R., Staufenbiel, M., Van Ginneken, C., Van Meir, F. & De Deyn, P.P. (2003) Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. *Eur J Neurosci*, **17**, 388-396.
- van Dooren, T., Dewachter, I., Borghgraef, P. & van Leuven, F. (2005) Transgenic mouse models for APP processing and Alzheimer's disease: early and late defects. *Subcell Biochem*, **38**, 45-63.
- Vloeberghs, E., Van Dam, D., Coen, K., Staufenbiel, M. & De Deyn, P.P. (2006) Aggressive Male APP23 Mice Modeling Behavioral Alterations in Dementia. *Behav Neurosci*, **120**, 1380-1383.
- Vloeberghs, E., Van Dam, D., Engelborghs, S., Nagels, G., Staufenbiel, M. & De Deyn, P.P. (2004) Altered circadian locomotor activity in APP23 mice: a model for BPSD disturbances. *Eur J Neurosci*, **20**, 2757-2766.
- Walsh, D.M. & Selkoe, D.J. (2007) Abeta Oligomers - a decade of discovery. *J Neurochem*, **101**, 1172-1184.
- Wirhth, O., Breyhan, H., Schafer, S., Roth, C. & Bayer, T.A. (2007) Deficits in working memory and motor performance in the APP/PS1ki mouse model for Alzheimer's disease. *Neurobiol Aging*, **Jan 8**; [Epub ahead of print].
- Wirhth, O., Weis, J., Kaye, R., Saido, T.C. & Bayer, T.A. (2006) Age-dependent axonal degeneration in an Alzheimer mouse model. *Neurobiol Aging*, **Sep 8**; [Epub ahead of print].
- Xie, S., Xiao, J.X., Gong, G.L., Zang, Y.F., Wang, Y.H., Wu, H.K. & Jiang, X.X. (2006) Voxel-based detection of white matter abnormalities in mild Alzheimer disease. *Neurology*, **66**, 1845-1849.
- Zhuo, J.M., Prescott, S.L., Murray, M.E., Zhang, H.Y., Baxter, M.G. & Nicolle, M.M. (2006) Early discrimination reversal learning impairment and preserved spatial learning in a longitudinal study of Tg2576 APPsw mice. *Neurobiol Aging*, **28**, 1248-1257.

## **Acknowledgments**

We thank the Sanofi-Aventis Neurodegenerative Disease Group for the generous gift of the animals that were involved in this study. We also want to acknowledge Maryvonne Guégan for her contribution to histological studies and Kyle Smith for carefully reading this manuscript. B. Delatour was supported by the Aging ATC (INSERM), the Fédération pour la Recherche sur le Cerveau, the Del Duca Foundation, and the ACI Neurosciences (French Research Department).

## TABLES

TABLE I - SENSORY FUNCTIONS

	WT	APP
<b>Buried food test</b> - <i>Latency to find the cracker (s)</i>		
3 months	200.2 ± 36.1	167.7 ± 21.3
6 months	153.9 ± 15.3	220.8 ± 34
15 months	177.8 ± 28.7	173.1 ± 26.3
<b>Visual cliff test</b> - <i>Proportion of steps down in the safe area</i>		
3 months	82%	81%
6 months	72%	73%
15 months	82%	77%

TABLE II - MOTOR FUNCTIONS

	WT	APP	
<b>Wire grid test- Latency to fall (s)</b>			
3 months	57.6 ± 2.4	41.7 ± 3.8	*
6 months	48.1 ± 4.3	40.1 ± 3.7	
15 months	43.9 ± 4.6	20.6 ± 4.2	*
<b>Coat hanger test - Reflex latency (s)</b>			
3 months	9.5 ± 1.4	11.9 ± 1.1	
6 months	16 ± 1.7	19.3 ± 1.3	
15 months	8.8 ± 0.9	8.4 ± 0.8	
<b>Coat hanger test - Latency to fall (s)</b>			
3 months	21.8 ± 1	20.4 ± 0.8	
6 months	19.4 ± 1.2	18.1 ± 0.9	
15 months	14.8 ± 1.3	9 ± 0.8	*
<b>Stationary beam test - Nb crossed segments</b>			
3 months	29.9 ± 5.6	27.9 ± 3.3	
6 months	37.1 ± 5.6	29.5 ± 3.1	
15 months	29.8 ± 4.6	8.4 ± 1.6	*
<b>Stationary beam test – Latency to fall (s)</b>			
3 months	42.7 ± 3.9	50.7 ± 2.2	
6 months	42.8 ± 4.2	51.8 ± 1.8	
15 months	47.4 ± 4.9	30.1 ± 2.9	*

TABLE III - LOCOMOTION & ANXIETY

	WT	APP	
<b>Openfield test - Horizontal activity (square crossings)</b>			
3 months	535.4 ± 20.5	452.6 ± 17.4	*
6 months	483.6 ± 38.9	353.1 ± 19.1	*
15 months	518.9 ± 39.8	305.2 ± 14.5	*
<b>Openfield test - Vertical activity (number of rearings)</b>			
3 months	42.2 ± 2.3	37.2 ± 2.2	
6 months	31.6 ± 3	25.9 ± 2.6	
15 months	26.2 ± 2	26.5 ± 2.3	
<b>Elevated +Maze test - Time spent in closed arms (s)</b>			
3 months	192.8 ± 6.8	179.2 ± 3.9	
6 months	183.1 ± 8.8	177.9 ± 7.1	
15 months	202.9 ± 8.6	222.3 ± 5	*
<b>Elevated +Maze test – Ratio NbO/Nb(O+C)</b>			
3 months	0.37 ± 0.01	0.39 ± 0.01	
6 months	0.37 ± 0.02	0.35 ± 0.02	
15 months	0.26 ± 0.03	0.24 ± 0.01	

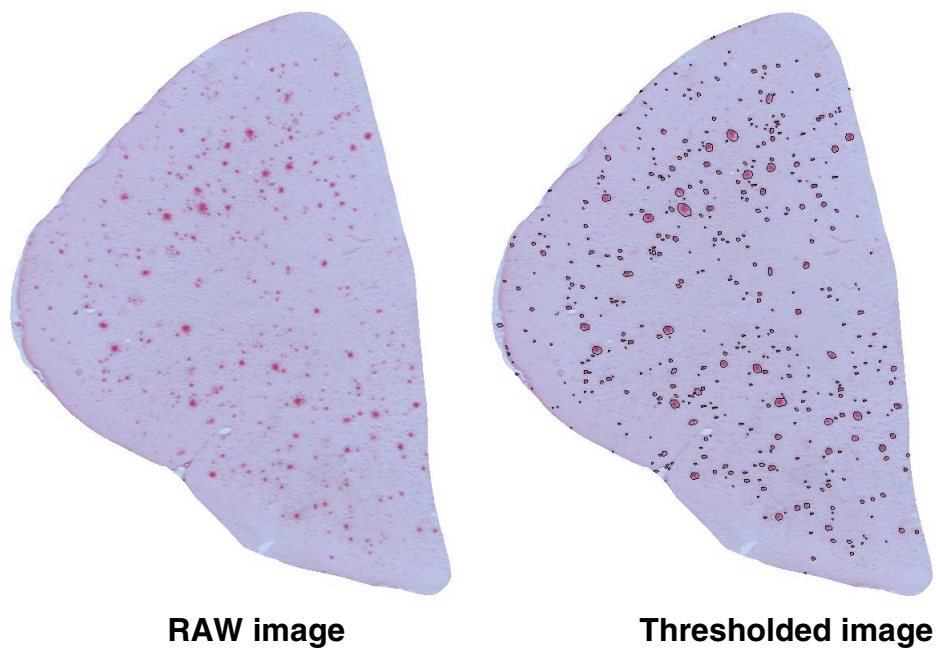
TABLE IV - MORPHOLOGICAL MEASURES

	WT	APP	
<b>Body weights (g)</b>			
3 months	26.2 ± 0.3	23.3 ± 0.2	*
6 months	33.1 ± 0.6	28.7 ± 0.3	*
15 months	36.9 ± 0.7	29.4 ± 0.2	*
<b>Neuropathology</b>			
Brain weight (mg)	482.9 ± 5.5	4490.3 ± 5.2	
Brain length (mm)	6.8 ± 0.1	7.1 ± 0	*
Amyloid load-whole brain (%)		1.58 ± 0.05	
Amyloid load-cortex (%)		2.57 ± 0.07	
Amyloid load-motor cortex (%)		4.02 ± 0.12	
Amyloid load-amygdala (%)		2.51 ± 0.19	

# FIGURES

FIGURE 1

(a)



(b)

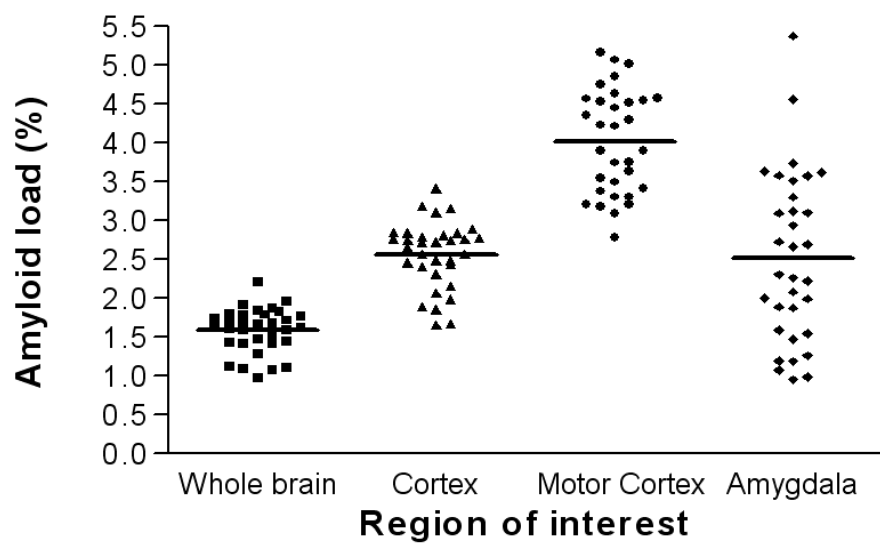




FIGURE 2

