Characterization of AD-like phenotype in aged APPSwe/PS1dE9 mice

Huang Huang · Sipei Nie · Min Cao · Charles Marshall ·
Junying Gao · Na Xiao · Gang Hu · Ming Xiao

Received: 28 April 2016 / Accepted: 12 July 2016 / Published online: 21 July 2016
© American Aging Association 2016

Abstract Transgenic APPSwe/PS1dE9 (APP/PS1) mice that overproduce amyloid beta ($A\beta$) are extensively used in the studies of pathogenesis and experimental therapeutics and new drug screening for Alzheimer's disease (AD). However, most of the current literature uses young or adult APP/PS1 mice. In order to provide a broader view of AD-like phenotype of this animal model, in this study, we systematically analyzed behavioral and pathological profiles of 24-month-old male APP/PS1 mice. Aged APP/PS1 mice had reference memory deficits as well as anxiety, hyperactivity, and social interaction impairment. Consistently, there was obvious deposition of amyloid plaques in the dorsal hippocampus with decreased expression of insulin-degrading enzyme, a proteolytic enzyme responsible for degradation of intracellular $A\beta$. Furthermore, decreases in hippocampal volume, neuronal number and synaptophysin expression, and astrocyte atrophy were also observed in aged APP/PS1 mice. This finding suggests that aged

APP/PS1 mice can well replicate cognitive and noncognitive behavioral abnormalities, hippocampal atrophy, and neuronal and astrocyte degeneration in AD patients, to enable more objective and refined preclinical evaluation of therapeutic drugs and strategies for AD treatment.

Keywords Aged · Alzheimer's disease · APP/PS1 mice · β -amyloid · Hippocampal atrophy · Noncognitive abnormalities

Introduction

Alzheimer's diseases (AD) is the most common neurodegenerative disease among the elderly, characterized by progressive memory loss and declining cognitive function, in addition to a variety of psychiatric and behavioral abnormalities (Holtzman et al. 2011). The pathogenesis of AD remains unclear and may likely be attributed to the combined effects of numerous factors, such as hereditary, environment, lifestyle, metabolic disturbance, and brain trauma (Querfurth and Laferla 2010; Jiang et al. 2013). However, aging is a significant risk factor for the onset of AD (Ballard et al. 2011; Bernick et al. 2012; Langbaum et al. 2013). Because of improvements in quality of life and health care, there has been a dramatic rise in human life expectancy. The total number of individuals and the proportion of aged population with AD are increasing year by year, causing tremendous social, financial, and public health burdens.

H. Huang · S. Nie · M. Cao · C. Marshall · J. Gao ·
N. Xiao · G. Hu · M. Xiao (✉)
Jiangsu Key Laboratory of Neurodegeneration, Nanjing Medical
University, 101 Longmian Avenue, Jiangning District, Nanjing,
Jiangsu 211166, China
e-mail: mingx@njmu.edu.cn

H. Huang
Department of Neurology, Sir Run Run Shaw Hospital, Nanjing
Medical University, Nanjing, Jiangsu 211166, China

C. Marshall
Department of Rehabilitation Sciences, University of Kentucky
Center of Excellence in Rural Health, Hazard, KY 41701, USA

Therefore, it is crucial to explore the interacting mechanisms between advancing age and AD.

Early-onset AD is caused primarily by mutations in the presenilin (PS)1, PS2, and/or A β precursor protein (APP) genes, while late onset AD may be associated with apolipoprotein E alleles (Bertram and Tanzi 2008; Rademakers and Rovelet-Lecrux 2009; Ferencz and Gerritsen 2015). Nevertheless, both types of AD show similar A β -related neuropathological changes (Huang and Mucke 2012). Correspondingly, various transgenic rodent models, such as those overexpressing mutant human APP or with both PS1 and tau, have been developed, providing valuable insights into specific aspects of AD pathogenesis (Bales 2012; Braidy et al. 2012; Bilkei-Gorzo 2014; Webster et al. 2014). Among these, APP/PS1 double transgenic mice are an extensively used model, showing progressive age-related development of A β accumulation and cognitive deficits (Jankowsky et al. 2001; Trinchese et al. 2004; van Groen et al. 2006; Meyer-Luehmann et al. 2009; Filali et al. 2011; Janus et al. 2015). Furthermore, previous studies have revealed positive results, such as demonstrating various antioxidants (Garcia-Alloza et al. 2010; Varamini et al. 2014; Yanagisawa et al. 2015), neuroprotective agents (Kilgore et al. 2010; Peng et al. 2012; Li et al. 2014), anti-neuroinflammation (Cherry et al. 2015; Guo et al. 2015), immune therapy (Sudduth et al. 2013; Carrera et al. 2013; Zhang et al. 2015), and nonpharmacological interventions (Liu et al. 2011; Jankowsky et al. 2005), which can attenuate or even reverse AD-like pathology in APP/PS1 mice. Unfortunately, none of these potentially useful results have been able to be reproduced in clinical therapies for AD patients. Thus, transgenic mice including APP/PS1 mice as a common tool for the study of AD, demonstrate an extremely low translational value from basic research to the clinical utilization (Zahs and Ashe 2010; Webster et al. 2014).

As an irreversible neurodegenerative disease, AD patients often pass away within 10–20 years of disease onset (Tanzi and Bertram 2008). Contrary to humans, the overall survival rate of transgenic AD mice is similar to that of the wild-type (WT) littermate controls, but the underlying mechanism for this discrepancy remains unclear (Zahs and Ashe 2010; Webster et al. 2014). Furthermore, to save experimental time and costs, most studies select young or adult transgenic mice, leaving out elderly subjects, as research participants (Bales 2012; Braidy et al. 2012; Bilkei-Gorzo 2014). This is

inconsistent with the fact that AD is essentially an age-related disease. The largely overwhelmingly positive results obtained when testing novel therapies in young or adult transgenic AD mice have failed to translate into similar positive clinical outcomes. Therefore, to provide theoretical basis for the rational use of transgenic AD models, it is necessary to systematically analyze their behavior and pathological phenotypes in old age. In this study, 24-month-old APP/P1 mice were used to assess AD-like pathology. The results suggested that aged APP/PS1 mice can better model pathophysiological features seen in human AD, so that may increase the success rate for therapeutic drugs and strategies advancing into clinical treatment of AD.

Materials and methods

Animals and housing

APP^{Swe}/PS1^{dE9} (APP/PS1) mice (Jankowsky et al. 2001) overexpressing chimeric mouse and/or human-mutated APP with Swedish mutation (APP^{695Swe}), and mutant human presenilin 1 with exon 9 deletion (PSEN1^{dE9}) genes, were used in the study. Postnatal day 28 male mice were weaned and separated from their female littermates and housed (two to four mice per cage) under standard laboratory conditions (temperature- and humidity-controlled conditions with a 12-h light-dark cycle with food and water ad libitum) until 24 months of age. There were no observable differences in mortality rates between APP/PS1 mice and wild-type (WT) mice in this study. A general health check was performed with all mice before each testing to ensure that behavioral findings were not the result of deteriorated physical conditions of the animals.

All experiments were conducted in accordance with international standards on animal welfare and the guidelines of the Institute for Laboratory Animal Research of Nanjing Medical University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Y-maze test

The Y-maze test was performed to measure working memory, as previously described (Wang et al. 2013). In brief, the Y-maze test contains two 5-min stages with an interval of 2 h. During the first stage, one arm, the

novel arm, was blocked by a black baffle, and mice were allowed to move freely within the other two arms. During the second stage, the novel arm was opened and mice could freely move throughout three arms. The percentage of time traveled in the novel arm, number of entries into the novel arm, and total distance traveled, as well as traveling speed, were counted.

Open field test

The open field test was carried out to measure exploration and anxiety-related behaviors as described (Chen et al. 2006). The open field box consisted of four identical chambers ($60 \times 60 \times 25$ cm), with an outlined center area (30×30 cm). Each animal was placed in the middle of chamber, which served as a starting point, then allowed to move freely for 10 min within the box. The time spent in the center area and number of entries into the center area were measured. The total distance traveled and mean speed of movement during the test were also counted.

Elevated plus maze

The elevated plus maze test was utilized to assess anxiety-related behavior in response to a potentially dangerous environment (Webster et al. 2013). The maze consists of two enclosed arms ($50 \times 10 \times 40$ cm), two open arms (50×10 cm), and center area (10×10 cm) elevated 100 cm above the floor. Each mouse was placed in the center of the maze, and the time spent in the open arms and number of entries into the open arms were analyzed.

Social interaction and memory test

Social interaction behavior was evaluated in an open field apparatus made of clear Plexiglas ($40 \times 40 \times 30$ cm). The procedure for the social interaction test included three phases: habituation, sociability test, and social memory test, as previously described (Lo et al. 2016). During the habituation phase, each of the two side chambers contained an inverted empty wire cup. Each mouse was given 10 min to explore the environment freely. The mice were habituated for 2 days. During the sociability phase, an unfamiliar mouse (stranger 1) was enclosed in one of the wire cup in a side chamber. The location of the unfamiliar mouse alternated

between the two side chambers across test. During the social memory phase, a new unfamiliar mouse (stranger 2) was enclosed in the wire cup that had been empty during the sociability phase. Exploring novelty was defined as when a test mouse climbed on the cup or oriented toward the cup with the distance between the nose and the cup less than 1 cm. The time spent in each chamber, and exploration of novel mice or empty cups, were recorded for the first 4 min of each session. All stranger mice were familiarized to being enclosed in inverted wire cups for 15 min daily on two consecutive days prior to the experiment.

Mouse activity in the above behavioral apparatuses was collected by a digital video camera connected with a computer-controlled system (Beijing Sunny Instruments Co. Ltd., China). All tests were performed by two independent experimenters who were blind to the treatment schedule.

Section preparation

Following deep anesthesia, mice were transcardially perfused with PBS followed by 4 % buffered formalin. Brains were dissected in the mid-sagittal plane, postfixed overnight at 4 °C, and embedded in paraffin following standard procedures. Both paraffin embedded half-brains were serially cut into sagittal sections at 5 μ m and divided into five sets.

Immunohistochemistry

Immunohistochemical staining was performed as previously described (Xu et al. 2013). After deparaffinization and rehydration, sections were incubated overnight at 4 °C with one of primary antibodies listed in Table 1 and incubated with secondary antibody for 1.5 h at room temperature. The reaction was visualized with DAB (Sigma-Aldrich).

Image analysis

The sets (every 25th sections, about 12–16 sections per mouse) of dorsal hippocampus stained with Cresyl violet, Thioflavin-S, or antibodies listed in Table 1 were photographed at $\times 100$ magnification using a digital microscope (Leica Microsystems). Individual images were exported to Image-Pro Plus 6.0 Analysis System (Media Cybernetics Inc., San

Table 1 Antibodies used in the present studies

Antibodies	MW (≈kDa)	Clone	Dilution	Source	Catalog no.
ADAM10	82	Rabbit, polyclonal	1:500; 1:1000 ^a	Wako	AB19026
BACE1	70	Mouse, monoclonal	1:1000; 1:2000 ^a	Millipore	MAB5308
β-tubulin	55	Mouse, monoclonal	1:1000 ^a	CWBio	CW0098
GFAP	51	Mouse, monoclonal	1:1000	Millipore	AB3594
Iba-1	20	Rabbit, polyclonal	1:500	Wako	019-19741
IDE	118	Rabbit, polyclonal	1:500; 1:1000 ^a	Abcam	ab32216
NeuN	34	Mouse, monoclonal	1:1000	Abcam	AB377
NEP	86	Rabbit, polyclonal	1:500; 1:1000 ^a	Millipore	AB4348
PS1	45	Rabbit, polyclonal	1:500; 1:1000 ^a	Sigma	PRS4203
SYP	70	Mouse, monoclonal	1:1000; 1:1500 ^a	Abcam	ab64581

^aFor Western blot

Francisco, CA, USA). The estimates of hippocampal volume were performed on serial Nissl-stained sections. The boundary of hippocampus and gray region that consists of pyramidal cell layer and granular cell layer was manually delineated. The total hippocampal area and gray matter area per section was per section were measured. Total hippocampal or gray matter volume per mouse was also calculated according to the principles of Cavalieri basic (Pereda-Pérez et al. 2013). The hippocampal white matter volume was obtained by the total hippocampal volume subtracting the hippocampal gray matter volume. The total neuron number (T) in the pyramidal layer of CA1 and granular cell layer of the dentate gyrus was quantified on NeuN-stained sections using the formula: $T = (N \times V)/t$. Where N , V , and t are the cell density, volume of the structure, and thickness of the section, respectively (Beauquis et al. 2014).

For quantitative analysis of Aβ plaque load, thioflavin-S, or 6E10-positive signals were determined by a standardized region of interest grayscale threshold analysis (Kim et al. 2009). The percentage of area occupied by thioflavin-S or 6E10 in the whole hippocampus, gray matter area, and white matter area was measured. In addition, expression levels of disintegrin and metalloproteinase 10 (ADAM10), β-site amyloid precursor protein-cleaving enzyme 1 (BACE1), PS1, neprilysin (NEP), insulin degrading enzyme (IDE), and synaptophysin (SYP) in the hippocampus or gray/white subareas was

assessed by measurement of mean integrated optical density (MIOD = IOD/total area), respectively (Xu et al. 2013).

Glial fibrillary acidic protein (GFAP) or ionized calcium-binding adaptor molecule 1 (Iba-1) immunostained in the stratum radiatum of CA1 was semi-quantified as previously described (Calvo-Ochoa et al. 2014). Briefly, only cells with clear cell bodies and complete processes were counted and expressed as the number of cells per square millimeter. The mean area of each GFAP-positive astrocytes and Iba-positive microglia was also measured. All quantification was done blind to animal genotype.

Western blot

Hippocampal tissues were homogenized and centrifuged at 4 °C and 12,000 rpm for 15 min. The samples were resolved on SDS-PAGE, transferred onto PVDF membranes using a Bio-Rad miniprotein-III wet transfer unit, then blocked with 5 % skim milk dissolved in TBST (pH 7.5, 10 mM Tris-HCl, 150 mM NaCl, and 0.1 % Tween 20) at room temperature for 1 h. Membranes were probed at 4 °C overnight with one of the primary antibodies listed in Table 1. Horseradish peroxidase-conjugated secondary antibodies (Vector Laboratories, Burlingame, CA, USA) were used, and bands were visualized using ECL plus detection system. β-tubulin was used as an internal control for protein loading and transfer efficiency.

Statistical analysis

All results were expressed as means $\pm$ SEM. The data were analyzed by two-tailed Student's *t* test. A value of $P < 0.05$ was considered statistically significant.

Results

Memory decline in aged APP/PS1 mice

APP/PS1 mice showed working memory impairment in the Y-maze test, as revealed by decreased time spent in the novel arm ($P = 0.042$; Fig. 1a) and the number of entries into the novel arm ($P = 0.005$; Fig. 1b). In addition, neither movement distance nor speed was significantly different between APP/PS1 mice and WT mice (both $P > 0.05$; Fig. 1c, d).

Increased anxiety-like behaviors in aged APP/PS1 mice

APP/PS1 mice displayed increased levels of general anxiety in the open field test, as shown by decreased time spent ($P = 0.005$; Fig. 2a) in the center area, as well as the number of crosses into the center area ($P = 0.028$; Fig. 2b). APP/PS1 mice also exhibited mild hyperactivity with a tendency to increase movement distance and speed, compared with WT controls (both $P > 0.05$; Fig. 2c, d).

The elevated plus maze test confirmed anxiety-like behaviors in APP/PS1 mice. They exhibited decreases in total movement time ($P = 0.017$; Fig. 3a) and distance ($P = 0.005$; Fig. 3b) in the open arm and number of entries into the open arm ($P = 0.028$; Fig. 3c). APP/PS1 mice showed a slightly longer movement distance and high movement speed compared with controls (both

$P > 0.05$) but with high individual variability in the both genotypes (Fig. 3d).

Social preference and memory impairment in aged APP/PS1 mice

Social preference tests results show that WT mice preferred to enter into the compartment housing a stranger mouse ($P = 0.047$, Fig. 4a), with a preference for exploring the novel mouse over the empty cup ($P = 0.003$; Fig. 4b). Alternatively, APP/PS1 mice were remained in the empty compartment ($P = 0.001$; Fig. 4a) and displayed no exploring preference between the novel mouse (stranger 1) and empty cup ($P = 0.501$; Fig. 4b). Social memory test results show that WT mice preferred to enter the compartment housing a new stranger mouse (stranger 2) compared with that housing stranger 1 ($P = 0.034$; Fig. 4c). By contrast, APP/PS1 mice did not show clear preference for staying in the chamber containing stranger 2 ($P = 0.398$; Fig. 4c). In addition, both groups showed preference for exploring stranger 2 over stranger 1 ($P = 0.004$ for WT mice; $P = 0.034$ for APP/PS1 mice), but contact time with stranger 2 was much longer in WT mice than APP/PS1 mice ($P = 0.013$; Fig. 4d).

Hippocampal atrophy and neuronal and synapse protein loss in aged APP/PS1 mice

Hippocampal atrophy and marked neuronal loss are the pathological basis for cognitive dysfunction and psychological symptoms of AD patients (Caselli and Reiman 2013; Schröder and Pantel 2016) but do not occur in young/adult APP/PS1 mice (Bilkei-Gorzo 2014; Webster et al. 2014). Therefore, we investigated whether these pathological hallmarks were able to be

Fig. 1 Y-maze test. **a** Time spent in the novel arm (NA). **b** The number of entries into the NA. **c** The total traveling distance during the test. **d** The mean traveling speed. Data represent means $\pm$ SEM from nine mice per group. * $P < 0.05$ vs. WT mice

Fig. 2 The open field test. **a** Time spent in the center area. **b** The number of entries into the center area. **c** The total traveling distance during the test. **d** The mean traveling speed. Data represent means ± SEM from nine mice per group. * $P < 0.05$ vs. WT mice

observed in aged APP/PS1 mice. Quantitative analysis of HE-stained hippocampal serial sections (Fig. 5a) revealed that aged APP/PS1 mice had a smaller dorsal hippocampus than their littermate controls, with significant reduction in section numbers ($P = 0.032$; Fig. 5b) and hippocampal volume ($P = 0.004$; Fig. 5c). Notably, white matter volume decreased more than that of the gray matter, the location of cellular bodies of pyramidal and granular neurons ($P = 0.005$; Fig. 5d).

Immunohistochemistry for neuronal marker NeuN followed by unbiased stereology analysis was performed to estimate the number of hippocampal neurons. The number of NeuN-positive hippocampal neurons in the CA1 and dentate gyrus of APP/PS1 mice was reduced to 86.6 and 79.6 %, respectively, compared with WT mice ($P = 0.041$, $P = 0.033$, respectively; Fig. 5e, f). Furthermore, both semi-quantitative immunohistochemistry (Fig. 5g, h) and Western blot analysis (Fig. 5i, j) demonstrated that hippocampal expression levels of synaptic marker SYP were reduced to 35 and 50 %, respectively (both $P < 0.001$). This indicates that

synaptic loss was more severe than neuronal loss in the hippocampus of this AD model.

A β plaque deposition and expression of A β metabolism-related enzymes in the hippocampus of aged APP/PS1 mice

Both 6E10 immunostaining (Fig. 6a) and thioflavin-S fluorescent staining (Fig. 6b) displayed a large number of A β plaques, predominately large-sized plaques, were present in the hippocampus and cerebral cortex of aged APP/PS1 mice. Furthermore, consistent with more severe atrophy of the hippocampal white matter, quantitative data revealed that the percentage area occupied by thioflavin-S or 6E10-labeled plaques was higher in the white matter than the gray matter (both $P < 0.001$; Fig. 6c).

It is known that brain A β accumulation is a consequence of the imbalance between A β generation and A β clearance (Tanzi and Bertram 2008). A β is cleaved from its precursor protein APP by

Fig. 3 The elevated plus maze test. **a** Time spent in the open arm. **b** The number of entries into open arm. **c** The total traveling distance during the test. **d** The mean traveling speed.

Data represent means ± SEM from nine mice per group. * $P < 0.05$ vs. WT mice

Fig. 4 Social interaction test and social memory test. **a** Time spent in each chamber in social interaction test. **b** Time spent exploring novel mice or empty cups. **c** Time spent in each chamber during the social memory test. **d** Time spent in exploring novel mouse 1 (stranger 1) or novel mouse 2 (stranger 2). Data represent means $\pm$ SEM from nine mice per group. * $P < 0.05$ vs. WT mice; # $P < 0.05$ vs. empty chamber. & $P < 0.05$ vs. novel mouse 1

β -secretase and γ -secretase, but it is most precluded by α -secretase to generate neuroprotective and soluble APP α fragments (sAPP α) (Morishima-Kawashima 2014). Neprilysin (NEP) and insulin-degrading enzyme (IDE) are responsible for proteolytic degradation of A β peptides from brain parenchyma (Tanzi et al. 2004; Tarasoff-Conway et al. 2015). Therefore, we determined whether different A β burden between the gray matter and white matter was associated with different expression patterns of these A β metabolism-related enzymes. Within the cell bodies of both pyramidal and granular neurons of aged APP/PS1 mice, there was high expression of α -secretase, IDE and NEP, but low expression of β -secretase and γ -secretase. In contrast, there was low expression of α -secretase, IDE and NEP, but high expression of β -secretase and γ -secretase in the white matter (Fig. 6d). The semi-quantitative analysis confirmed that the gray matter and white matter were differences in expression levels of α -secretase ($P < 0.001$), β -secretase ($P = 0.003$), γ -secretase ($P < 0.001$), IDE ($P < 0.001$), and NEP ($P < 0.001$) (Fig. 6e). Western blot revealed that the expression levels of α -, β -, and γ -secretases and NEP were similar between aged APP/PS1 mice and WT mice (all $P > 0.05$), but IDE levels

significantly decreased ($P = 0.036$) in the hippocampal neurons of aged APP/PS1 mice (Fig. 6f, g).

Astrocyte atrophy in the hippocampus of aged APP/PS1 mice

Expression and distribution of astrocytes in the hippocampus was observed by immunohistochemical staining for GFAP. In the hippocampus of aged APP/PS1 mice, there were obvious GFAP-positive signals around A β plaques, but astrocytes lacked normal morphology. Astrocytes also underwent apparent atrophy in the brain parenchyma distant to the A β plaques. By contrast, a large number of GFAP-positive astrocytes were present throughout the hippocampus of WT controls, with most being of the activated phenotype characterized by hypertrophic cellular bodies and processes (Fig. 7a). Astrocyte loss and atrophy in the hippocampus of APP/PS1 mice was confirmed by quantitative analyses revealing marked decreases in GFAP-positive cell number ($P = 0.037$; Fig. 7b) and cell area ($P = 0.019$; Fig. 7c). Therefore, similar to neuronal elements, astrocytes also underwent degeneration during the later stages of A β pathology in APP/PS1 mouse model. In addition, activated Iba-1-

Fig. 5 Analyses of hippocampal volume, neuronal number and synaptic protein expression. **a** Series of Nissl-stained sagittal brain sections showing that the cross area of the dorsal hippocampus was smaller in 24-M aged APP/PS1 mice than WT controls. Note from no. 275 section (about 1.66 mm lateral to the mid-sagittal fissure), the intermediate region of the hippocampus, a transitional zone of the dorsal hippocampal region, and ventral hippocampal region, was observed in APP/PS1 mice but was not present in WT mice until at no. 305 section. Scale bar = 500 μ m. **b** Counting the total number of sections that contain the dorsal hippocampus. **c** Quantification of the volume of the dorsal hippocampus. **d** Relative volume of the pyramidal (Py) and granular (Gr) layers (gray matter) and other layers (white matter) between APP/PS1 mice

and WT controls. **e** Representative microimages of neuron-specific nuclear protein (*NeuN*) immunopositive neurons in the hippocampus CA1 of APP/PS1 mice and WT controls. **f** Unbiased stereological counting of NeuN-positive neurons in the hippocampus of the two genotypes. **g** Representative microimages of synaptophysin (*SYP*) immunoreactivity in the hippocampus of APP/PS1 mice and WT controls. **h** The quantification analysis of the mean integrated optical density (*MIOD*) of *SYP*. **i, j** Western blotting and densitometry analysis of *SYP* expression in the hippocampus of the two genotypes. Data represent means $\pm$ SEM. Five mice per group for pathological analyses and three mice per group in three independent Western blotting analysis experiments. * $P < 0.05$ vs. WT controls; # $P < 0.05$ vs. other hippocampal layers

positive microglia were observed in the hippocampus of both genotypes, but many amoeba-like microglia were present around A β plaques (Fig. 7a). Quantitative analysis revealed a high number of microglia in the hippocampus of APP/PS1 mice, suggesting the occurrence of long-term chronic inflammation ($P = 0.042$; Fig. 7c).

Lower BDNF levels in the hippocampus of aged APP/PS1 mice

Brain-derived neurotrophic factor (BDNF) is a central regulator of hippocampal neuron survival and synaptic plasticity (Lipsky and Marini 2007). Levels of BDNF have been reported to be decreased in the preclinical

Fig. 6 Analyses of amyloid plaque load and expression of Aβ metabolism enzymes in the hippocampus. **a**, **b** Brain sections from 24-M aged APP/PS1 mice and WT controls were stained with thioflavin-S (**a**) or immunostained with anti-6E10 antibody (**b**) to label plaques. Few plaques were present in the pyramidal (Py) layer and granular (Gr) layer. **c** Percentage of brain area occupied by thioflavin-S and 6E10-labeled Aβ deposition in the hippocampus. **d** Representative microimages of immunostaining for α-secretase (ADAM10), β-secretase (BACE1), γ-secretase (PS1), neprilysin (NEP), and insulin-degrading enzyme (IDE). High immunoreactivity of ADAM10, NEP, and IDE was observed in the Py layer, while expression of BACE1 and PS1 was relatively low.

In contrast, in the oriens (Or), radiatum (Rad), and lacunosum moleculare (Lmol) layers, high expression of BACE1 and PS1, but low expression of ADAM10, NEP, and IDE. **e** The quantification analysis of the mean integrated optical density (MIOD) of ADAM10, BACE1, PS1, NEP, and IDE in the Py plus Gr layers and other hippocampal layers. **f**, **g** Western blotting and densitometry analysis of ADAM10, BACE1, PS1, NEP, and IDE expression in the hippocampus of the two genotypes. Data represent means ± SEM from five mice per group for pathological analyses and from three mice per group in three independent Western blotting analysis experiments. **P* < 0.05 vs. Py+Gr (Fig. 6c) or WT mice (Fig. 6e and 6g)

stages of AD (Peng et al. 2005). Recent studies revealed that BDNF is associated with age-related decline in

hippocampal volume (Erickson et al. 2010) and prevents hippocampal atrophy in APP/PS1 mice

Fig. 7 Analyses of reactive gliosis in the hippocampus. **a** A considerable proportion of GFAP-positive astrocytes exhibited an activated shape (*arrow*), characterized by larger cell bodies in the hippocampus of 24-month-old WT mice. In contrast, dense GFAP immunoreactive products, lacking the normal morphology of astrocytes, were around A β plaques in age-matched APP/PS1 mice. Some astrocytes distant to the A β plaques only had few, short processes (*arrowhead*), indicating occurrence of atrophy. Activated Iba-1-positive microglia with long processes (*arrow*) were present in both APP/PS1 mice, although those around A β plaques became an amoeba-like shape and process disappearance (*arrowhead*). Activated microglia were also frequently observed in the hippocampus of aged WT mice. **b** Cell counts of GFAP-positive astrocytes and Iba-1-positive microglia. **c** Cellular surface of GFAP-positive astrocytes and Iba-1-positive microglia. Data represent mean $\pm$ SEM from five mice per group. * $P < 0.05$ vs. WT controls

(Rantamäki et al. 2013). Therefore, we determined whether BDNF was downregulated in the hippocampus of aged APP/PS1 mice. As expected, both semi-quantitative immunohistochemistry and Western blot demonstrated that aged APP/PS1 mice had lower levels of BDNF in the hippocampus than WT mice (both $P < 0.001$, Fig. 8a–d).

Discussion

Aging is the greatest risk factor for AD, occurring primarily in the 5th quintile of life. APP/PS1 transgenic mice expressing mutant human APP and PS1 genes are a useful research tool to study A β -related pathogenesis. There are several studies characterizing cognitive as well as noncognitive abnormality in APP/PS1 mice at 15–24 months of age (Morgan et al. 2000; Arendash et al. 2001; Sadowski et al. 2004; Wilcock et al. 2004; Hooijmans et al. 2007; Minkeviciene et al.

2008; Pistell et al. 2008; Mei et al. 2010; Ke et al. 2011; Gengler et al. 2012; Manczak and Reddy 2012; Wang et al. 2012; Duffy and Hölscher 2013; Do et al. 2014; Huang et al. 2015; Janus et al. 2015; Sahlholm et al. 2015; Yousefi et al. 2015) (A detailed summary is available in Table 2). However, the majority of experiments utilize young or adult APP/PS1 mice, which does not replicate typical hallmarks of AD, such as severe hippocampal atrophy and extensive neuronal loss (Bales 2012; Bilkei-Gorzo 2014). This shortcoming is then seen when positive therapeutic outcomes from APP/PS1 mice fail in clinical treatment on patients with AD (Bales 2012; Bilkei-Gorzo 2014). In order to provide a broader view of AD-like phenotype of this animal model, in this study, we systematically assessed behavioral and pathological changes of 24-month-old APP/PS1 mice. The results indicate that aged APP/PS1 mice could enable more objective and refined preclinical evaluation of therapeutic drugs and strategies for AD treatment.

Fig. 8 Analysis of BDNF expression in the hippocampus. **a** Representative microimages of BDNF immunoreactivity in the hippocampus of APP/PS1 mice and WT controls. **b** The quantification analysis of the mean integrated optical density (MIOD) of BDNF. **c, d** Western blotting and densitometry analysis of BDNF

expression in the hippocampus of the two genotypes. Data represent means $\pm$ SEM from five mice per group for pathological analyses and from three mice per group in three independent Western blotting analysis experiments. * $P < 0.05$ vs. WT controls

The progressive impairment of cognitive function is one typical clinical manifestation of AD. Previous studies report that APP/PS1 mice show reference memory impairments by 3 months of age and persist throughout life (Reiserer et al. 2007; Bernardo et al. 2009; O'Leary and Brown 2009). By comparing these results with our previous Y-maze test for behavioral analysis of 11–12-month-old APP/PS1 mice (Xu et al. 2013, 2015), we found that 24-month-old APP/PS1 cohorts show more severe working and reference memory impairment. This suggests that the 24-month-old APP/PS1 mouse model can imitate age and disease course-related cognitive decline of AD. In addition, we found that there is considerable individual variability in cognitive performance among aged APP/PS1 mice as well as normal aged mice. Further studies are necessary to reveal the underlying mechanism for individual heterogeneity of age-associated cognitive decline, which might be beneficial to find key targets for prevention and treatment of brain aging and AD.

Apart from progressive cognitive dysfunction, AD patients often display anxiety, apathy, emotional changes, and varying degrees of social interaction disability, but there is no clear correlation between noncognitive abnormalities and cognitive impairment (Deutsch and Rovner 1991; Apostolova et al. 2014). It is known that anxiety-related behavior often manifests prior to cognitive impairment in the longitudinal course of AD (Ramakers et al. 2013), a characteristic that also occurs in APP/PS1 mice. Previous studies reported that 3-month-old APP/PS1 mice exhibit anxiety-like phenotype, although without spatial cognitive dysfunction, when compared with age-matched WT controls

(Trinchese et al. 2004; Webster et al. 2014). The open field and elevated plus maze test confirmed that anxiety-like behaviors continue to exist in the late stages of this mouse AD model, similar to the Tg2576 (Gil-Bea et al. 2007; Lassalle et al. 2008), J20 (Harris et al. 2010; Cissé et al. 2011), and 5xFAD mice (Wirths et al. 2010; Shukla et al. 2013), all of which also exhibit anxiety-related behavior deficits in the elevated plus maze task. However, there is no anxiety-related behavior abnormality in the APP/PS1 double knock-in mice from 7 to 24 months old (Webster et al. 2013); while 3xFAD mice show decreased anxiety-related behavior in the open field and elevated plus maze test (Nelson et al. 2007; Filali et al. 2012). The variability of anxiety-related behavior in these commonly used AD mouse models supports a heterogeneous range of noncognitive symptoms caused by A β -related pathogenesis.

Furthermore, compared with younger cohorts, aged APP/PS1 mice display more advanced social behavioral abnormalities. Previous studies reported that 6-month-old APP/PS1 mice experience mild decline in social interaction function, exhibiting no preference to stay in the empty chamber versus the one having unfamiliar mouse (Zhang et al. 2013; Hsiao et al. 2014). The present study revealed that 24-month-old APP/PS1 mice spent more time in the empty chamber than with the unfamiliar partner, indicating the occurrence of social interaction impairment. Aged APP/PS1 mice also show impairment of social interaction memory, revealed by no preference to stay with the novel mouse versus the familiar one. In addition, similar to later stages of AD patients, aged APP/PS1 show mild hyperactivity, reflected by increased movement speed in the elevated

Table 2 The late Alzheimer-like pathophysiological analyses of APP/PS1 mice

Months	12–14	15–18	19–20	21–22	23–24
Y/T-maze test			Huang et al. (2015)		
Morris water maze test	Park et al. (2006)	Minkeviciene et al. (2008), Arendash et al. (2001)	Huang et al. (2015)	Sadowski et al. (2004)	Ke et al. (2011)
Radial arm water maze test	Xu et al. (2015)	Morgan et al. (2000), Arendash et al. (2001)			Wilcock et al. (2004)
Barnes maze test		O'Leary and Brown (2009)			
Open field test		Arendash et al. (2001)	Huang et al. (2015)		Ke et al. (2011)
Elevated plus maze test	Knafo et al. (2009)				
Fear conditioning test	Knafo et al. (2009)	Janus et al. (2015)			
Passive avoidance test		O'Leary and Brown (2009)			Ke et al. (2011)
Analysis of amyloid burden	Park et al. (2006), Xu et al. (2015), Knafo et al. (2009)	Minkeviciene et al. (2008), Sahlholm et al. (2015), Pistell et al. (2008), Mei et al. (2010), Gengler et al. (2012), Hooijmans et al. (2007), Janus et al. (2015), Morgan et al. (2000)	Huang et al. (2015), Duffy and Hölscher (2013)	Sadowski et al. (2004), Wang et al. (2012)	Ke et al. (2011), Manczak and Reddy (2012), Wilcock et al. (2004), Yousefi et al. (2015)
Analysis of neuronal loss	Knafo et al. (2009) ^a		Huang et al. (2015)	Sadowski et al. (2004)	
Analysis of spine or synapse loss	Park et al. (2006), Xu et al. (2015), Knafo et al. (2009)	Minkeviciene et al. (2008)	Huang et al. (2015)		
Analysis of reactive gliosis	Park et al. (2006), Xu et al. (2015)	Minkeviciene et al. (2008), Mei et al. (2010), Gengler et al. (2012)	Huang et al. (2015), Duffy and Hölscher (2013)		Ke et al. (2011)
Analysis of vascular volume or density		Do et al. (2014), Hooijmans et al. (2007)			
Analysis of neuroinflammatory factors	Xu et al. (2015)				
Analysis of neurotrophic factors	Park et al. (2006)				Ke et al. (2011)

Pathological and biochemical analyses are mainly investigated in the hippocampus and cerebral cortex. Blank space represents the time point that no data are available for the respective behavioral, pathological, or biochemical analysis

^a No difference compared with wild-type mice. Currently, there is no data addressing ≥ 25 -month-old APP/PS1 mice

plus maze test and open field. This is not observed in 6–18-month-old APP/PS1 mice (Lalonde et al. 2004; Cao et al. 2007; Sood et al. 2007). In addition to evident cognitive impairment, behavioral results show that 24-month-old APP/PS1 mice emulate other psychiatric and behavioral symptoms including anxiety, social behavior withdrawal, and hyperactivity.

During AD the hippocampus is one of the first regions of the brain to suffer damage. The mechanism of hippocampal atrophy in AD patients is not entirely clear and may be due to neuronal loss, as well as neurite and synaptic degeneration caused by A β , highly phosphorylated tau protein, and neurofibrillary tangles, together with other damage factors such as hypotension, diabetes, hyperlipidemia, epilepsy, stress, and affective disorders (Dhikav and Anand 2012; Roberts et al. 2014; Elcombe et al. 2015). Accumulated evidence shows that there is a close correlation between hippocampal atrophy and cognitive impairment in patients with AD (Yavuz et al. 2007; Dhikav and Anand 2011). Furthermore, hippocampal atrophy and dysfunction have also occurred in mild cognitive impairment (MCI) patients (van de Pol et al. 2007). Hippocampal volume reduction rate is an effective tool to help predict whether the MCI will develop into AD (Perrin et al. 2009; Nasrallah and Wolk 2014).

Conversely, various 6–12-month-old AD mouse models, including PDAPP, TG2576, APP23, APP/PS1, 5 $\times$ FAD, and 3 $\times$ Tg-AD do not show obvious hippocampal atrophy and neuronal loss (Bales 2012; Braidy et al. 2012; Bilkei-Gorzo 2014; Webster et al. 2014). Based on stereological analysis, we found that dorsal hippocampal volume and NeuN-positive neurons in 24-month-old APP/PS1 mice are reduced by 24.5 and 19.4 %, respectively (Fig. 5). These pathological changes suggest that aged APP/PS1 mice display hippocampal atrophy and neuronal loss, although this characteristic pathological change occurs in the early stages of AD patients (Schröder and Pantel 2016). Further research is needed to identify a precise beginning to the hippocampal atrophy and massive loss of neurons in APP/PS1 model, which will be helpful for objective preclinical evaluation of neuroprotective drugs for AD.

Excessive accumulation of neurotoxic A β peptides and subsequent formation of senile plaques in the brain parenchyma is the fundamental basis for the gradual, progressive development of AD pathology (Mawuenyega et al. 2010; Holtzman et al. 2011). An earlier study reported that diffuse amyloid plaques begin

to appear in the hippocampus and cerebral cortex of APP/PS1 mice at 3 months old (Trinchese et al. 2004). Subsequent studies have revealed an age-dependent pattern of A β burden in this AD model (Samaroo et al. 2012; Janus et al. 2015). By use of thioflavin-S and 6E10 immunostaining, we found that 24-month-old APP/PS1 mice versus their 20-month counterparts have a higher percentage of area occupied by thioflavin-S-labeled core plaques (7.24 vs. 3.15 %) or 6E10-labeled diffusing plaques (3.02 vs. 3.65 %) in the hippocampus (Huang et al. 2015). These results suggest that even during 20 to 24 months of age, senile plaques in APP/PS1 mouse brain are still in a dynamic change state.

Furthermore, as mentioned above, APP cleavage is controlled by both α -secretase-mediated nonamyloidogenic pathway and β - and γ -secretase-mediated amyloidogenic pathway (Morishima-Kawashima 2014). Previous studies revealed that young APP/PS1 mice have high levels of amyloidogenic enzyme PS1 (Morishima-Kawashima 2014). In contrast, we demonstrated that aged APP/PS1 mice have no changes in A β enzymes including ADAM10, BACE1, and PS1 in the hippocampus, compared with their littermates.

We hypothesized that the relative reduction of γ -secretase in the hippocampus of aged APP/PS1 mice could reduce the production of A β . This presumption is supported by the presence of only mature fibrillary plaques, without new inflammatory plaques, in the hippocampus of 24-month-old APP/PS1 mice. Similar to adult APP/PS1 mice, the deposition of A β plaques was mainly present in the molecular layer and stratum lacunosum moleculare, while being virtually absent in the pyramidal cell layer and the granule cell layer (Bas Orth et al. 2005; Oksman et al. 2006; Donkin et al. 2010; Scott et al. 2012; Xu et al. 2012, 2016). Furthermore, our previous and present results suggest that laminar heterogeneity and A β load is correlated with laminar variability in expression of APP secretases (Xu et al. 2016). As shown in Fig. 6, cellular bodies of pyramidal neurons express high levels of α -secretase, but low levels of β -secretase and γ -secretase. In contrast, dendrites of pyramidal cells located in the oriens, radiatum, and lacunosum moleculare layers display high expression of β -secretase and low expression of α -secretase. In addition, it has been shown that over-expression of human sAPP α ameliorates A β pathogenesis in APP/PS1 mice. Correspondingly, immunoneutralization of sAPP α by antibody causes hippocampal neuronal

apoptosis in this model (Obregon et al. 2012). These evidences, taken together, highly suggest that elevated α -secretase levels in neuronal cell bodies, give them the ability to produce neuroprotective sAPP α and maximally reduce neurotoxic A β production, in turn protects hippocampal neurons in the relatively long-term survival in AD-like pathology.

We also determined that human hippocampal neuronal cell bodies expressed high levels of β -secretase and γ -secretase but practically did not express α -secretase. This could potentially increase A β production, causing progressive self-death during the AD process (Xu et al. 2016). Different expression patterns of APP secretase in human and mouse neurons may contribute to a significant prolonging of the relative survival time of APP/PS1 mice compared with patients with AD. However, the intrinsic mechanism for species differences in neuronal susceptibility to A β pathogenesis needs further investigation.

It is known that brain A β homeostasis depends on the balance between the generation and clearance of A β (Tanzi and Bertram 2005). Aged APP/PS1 mice show decreases in IDE levels, without changes in A β -generating enzymes including ADAM10, BACE1, and PS1 in the hippocampus, compared with their WT littermate controls. This result indicates that there is decreased A β clearance, without increased A β production, in the late stage of this AD model. This phenotype is consistent with previous human studies that A β clearance is impaired in both early-onset and late-onset forms of AD (Mawuenyega et al. 2010; Potter et al. 2013). Based on this, it seems that aged APP/PS1 mice may be available for screening potential anti-AD agents targeting at A β clearance.

NEP and IDE are two key metabolic enzymes used in brain A β degradation, and each plays an important role in maintaining the homeostasis of brain A β . The primary effect of IDE is degradation of A β polymers and fibers (Eckman and Eckman 2005; Qiu and Folstein 2006), while NEP is used predominantly in degradation of A β monomers and oligomers (El-Amouri et al. 2008). Our results show that IDE content in the hippocampus of APP/PS1 mice clearly decreases, whereas NEP levels do not differ from those in WT controls. This result is also consistent with the feature of hippocampal A β load, wherein the percentage of area occupied by thioflavin-S-positive fibrillary plaques is higher than 6E10-immunopositive diffuse plaques. Unlike aged APP/PS mice, previous studies have reported that

the protein levels and activities of NEP are significantly decreased in the brain of patients with AD, while IDE is not significantly altered (Yasojima et al. 2001; Caccamo et al. 2012; Wang et al. 2005). Therefore, for AD patients, impaired A β clearance could be mainly due to decreased degradation of A β monomers and oligomers, whereas, for aged APP/PS1 mice, it is primarily attributed to reduced degradation of A β polymers and fibers. It is known that A β monomers and oligomers are the main components of neurotoxic substances in the brain, and the toxicity of amyloid plaques is relatively weak (Schnabel 2011). Therefore, we speculate that decreased IDE, but not NEP impairment, may be another key factor for the increased survival time of APP/PS1 mice when compared with patients with AD.

Astrocytes are responsible for maintaining the homeostasis of brain and neural network, by playing a variety of essential functions, including maintaining cerebral water, ions, pH, and neurotransmitter homeostasis, modulating cerebral microcirculation and synaptic transmission and producing neurotrophic factors and antioxidants (Allen and Barres 2009; Baxter 2012). Furthermore, recent studies, including from our laboratory, have demonstrated that astrocytes, via specific expression of the water channel aquaporin 4, are responsible for perivascular clearance of A β and other pathological proteins from the brain (Iliff et al. 2012; Xie et al. 2013; Arbel-Ornath et al. 2013; Xu et al. 2015). Astrocytes make rapid responses to numerous varieties of damage and pathological changes through a process referred to as reactive astrogliosis. Evidence of astrogliosis has been documented in early stages of AD (Schipper et al. 2006; Owen et al. 2009; Carter et al. 2012), whereas astrocyte death or atrophy in AD brains from patients and animal models has also been reported (Smale et al. 1995; Kobayashi et al. 2002, 2004). Extensive gliosis is present in the hippocampus of adult APP/PS1 mice (Xu et al. 2013, 2015). Whereas in the 24-month-old APP/PS1 mice, a large number of astrocytes undergo atrophy throughout the hippocampus and even death surrounding A β plaques. There is no doubt that degeneration of astrocytes affects their normal functions, including BDNF production, which subsequently exacerbates AD-like pathology in aged APP/PS1 mice. In addition, a large number of microglia were excessively activated, exhibiting amoeba-like shape in aged APP/PS1 mice. Although phagocytic microglia can facilitate A β degradation and clearance, protecting adjacent neuronal elements, they may also produce a

variety of inflammatory factors, causing neurotoxic effects (Derecki et al. 2014; Mhatre et al. 2015). Therefore, functional impairment of glial cells and microglia may act as a key player in the late stages of AD and protect their functions could serve as an effective strategy against moderate-to-severe AD (Osborn et al. 2016).

BDNF is important in neuronal growth and survival and synaptic processes of memory (Lipsky and Marini 2007). Evidence has suggested that dysfunction of BDNF is a possible contributor to the pathology and symptoms of AD (Tapia-Arancibia et al. 2008). BDNF is found to be decreased in APP/PS1 mice even in the early stages of AD-like pathology (Peng et al. 2009; Hsiao et al. 2014). Apart from BDNF, other neurotrophic factors, such as nerve growth factor (NGF) and glial cell-derived neurotrophic factor (GDNF), are also involved in normal aging and AD (Allen et al. 2013; Budni et al. 2015). It is a limitation of this study that we have only investigated BDNF levels in aged APP/PS1 mice.

In conclusion, via systematic behavioral and pathological analysis, our results have revealed that 24-month-old APP/PS1 mice can emulate various clinical features of AD patients, such as declines in the spatial memory and exploratory ability, and neuropsychiatric symptoms including anxiety and social behavior dysfunction. The mice also show A β plaque deposition and other similar pathological characteristics seen in AD patients, such as hippocampal atrophy, neuronal loss and severe glial cell atrophy, none of which occurs in adult APP/PS1 mice. In addition, when compared with AD patients, aged APP/PS1 mice have unique characteristics of AD-like pathology, including a considerable number of survival hippocampal neurons with high expressions of α -secretase and NEP. Nonetheless, it should be pointed out that we did not assess the long-term memory impairment of 24-month-old APP/PS1 mice, due to the fact that aged mice may be unable to tolerate high physical activity during the water maze training. To reduce the number of animals used, we also did not perform systematic evaluation of AD-like progress in this model by using APP/PS1 mice at different months of age. In addition, it will be interesting to determine whether APP/PS1 mice have an age-dependent relationship between hippocampal atrophy and A β accumulation, and the underlying mechanisms for the species difference between humans and mice in the sensitivity of the central nervous system to A β

neurotoxicity. These studies will contribute to a more comprehensive understanding of the AD-like phenotype of APP/PS1 mice, which will be beneficial for more a refined preclinical therapeutic evaluation of AD.

Acknowledgments This work was supported by the grants from the National Natural Science Foundation of China (30971020 and 30973517) and the Natural Science Foundation of Jiangsu Educational Department (09KJA310003).

References

- Allen NJ, Barres BA (2009) Neuroscience: glia—more than just brain glue. *Nature* 457(7230):675–677
- Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK (2013) GDNF, NGF and BDNF as therapeutic options for neurodegeneration. *Pharmacol Ther* 138(2):155–175
- Apostolova LG, Di LJ, Duffy EL, Brook J, Elashoff D, Tseng CH, Fairbanks L, Cummings JL (2014) Risk factors for behavioral abnormalities in mild cognitive impairment and mild Alzheimer's disease. *Dement Geriatr Cogn Disord* 37(5–6):315–326
- Arbel-Ornath M, Hudry E, Eikermann-Haerter K, Hou S, Gregory JL, Zhao L, Betensky RA, Frosch MP, Greenberg SM, Bacskai BJ (2013) Interstitial fluid drainage is impaired in ischemic stroke and Alzheimer's disease mouse models. *Acta Neuropathol* 126(3):353–364
- Arendash GW, King DL, Gordon MN, Morgan D, Hatcher JM, Hope CE, Diamond DM (2001) Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. *Brain Res* 891:42–53
- Bales KR (2012) The value and limitations of transgenic mouse models used in drug discovery for Alzheimer's disease: an update. *Expert Opin Drug Discov* 7(4):281–297
- Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (2011) Alzheimer's disease. *Lancet* 377(9770):1019–1031
- Bas Orth C, Vlachos A, Del Turco D, Burbach GJ, Haas CA, Mundel P, Feng G, Frotscher M, Deller T (2005) Laminar-specific distribution of Synaptopodin, an actin-associated molecule essential for the spine apparatus, in identified principal cell dendrites of the mouse hippocampus. *J Comp Neurol* 487(3):227–239
- Baxter P (2012) Astrocytes: more than just glue. *Dev Med Child Neurol* 54(4):291
- Beauquis J, Vinuesa A, Pomilio C, Pavía P, Galván V, Saravia F (2014) Neuronal and glial alterations, increased anxiety, and cognitive impairment before hippocampal amyloid deposition in PDAPP mice, model of Alzheimer's disease. *Hippocampus* 24(3):257–269
- Bernardo A, Harrison FE, McCord M, Zhao J, Bruchey A, Davies SS, Jackson Roberts L 2nd, Mathews PM Jr, Matsuoka Y, Ariga T, RK Y, Thompson R, McDonald MP (2009) Elimination of G β 3 synthase improves memory and reduces amyloid-beta plaque load in transgenic mice. *Neurobiol Aging* 30(11):1777–1791

- Bernick C, Cummings J, Raman R, Sun X, Aisen P (2012) Age and rate of cognitive decline in Alzheimer disease: implications for clinical trials. *Arch Neurol* 69(7):901–905
- Bertram L, Tanzi RE (2008) Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nat Rev Neurosci* 9(10):768–778
- Bilkei-Gorzo A (2014) Genetic mouse models of brain ageing and Alzheimer's disease. *Pharmacol Ther* 142(2):244–257
- Braid N, Muñoz P, Palacios AG, Castellano-Gonzalez G, Inestrosa NC, Chung RS, Sachdev P, Guillemin GJ (2012) Recent rodent models for Alzheimer's disease: clinical implications and basic research. *J Neural Transm (Vienna)* 119(2):173–195
- Budni J, Bellettini-Santos T, Mina F, Garcez ML, Zugno AI (2015) The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. *Aging Dis* 6(5):331–341
- Caccamo A, Oddo S, Sugarman MC, Akbari Y, LaFerla FM (2012) Age- and region-dependent alterations in Abeta-degrading enzymes: implications for Abeta-induced disorders. *Neurobiol Aging* 26(5):645–654
- Calvo-Ochoa E, Hernández-Ortega K, Ferrera P, Morimoto S, Arias C (2014) Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. *34(6):1001–1008*
- Cao D, Lu H, Lewis TL, Li L (2007) Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. *J Biol Chem* 282(50):36275–36282
- Carrera I, Etcheverría I, Li Y, Fernández-Novoa L, Lombardi V, Vigo C, Palacios HH, Benberin VV, Cacabelos R, Aliev G (2013) Immunocytochemical characterization of Alzheimer disease hallmarks in APP/PS1 transgenic mice treated with a new anti-amyloid- β vaccine. *Biomed Res Int* 2013:709145
- Carter SF, Schöll M, Almkvist O, Wall A, Engler H, Långström B, Nordberg A (2012) Evidence for astrocytosis in prodromal Alzheimer disease provided by 11C-deuterium-L-deprenyl: a multitracer PET paradigm combining 11C-Pittsburgh compound B and 18F-FDG. *J Nucl Med* 53(1):37–46
- Caselli RJ, Reiman EM (2013) Characterizing the preclinical stages of Alzheimer's disease and the prospect of presymptomatic intervention. *J Alzheimers Dis* 33(Suppl 1):S405–S416
- Chen H, Yang Y, Yao HH, Tang XC, Ding JH, Wang H, Hu G (2006) Protective effects of iptakalim, a novel ATP-sensitive potassium channel opener, on global cerebral ischemia-evoked insult in gerbils. *Acta Pharmacol Sin* 27(6):665–672
- Cherry JD, Olschowka JA, O'Banion MK (2015) Arginase 1+ microglia reduce A β plaque deposition during IL-1 β -dependent neuroinflammation. *J Neuroinflammation* 12:203
- Cissé M, Sanchez PE, Kim DH, Ho K, Yu GQ, Mucke L (2011) Ablation of cellular prion protein does not ameliorate abnormal neural network activity or cognitive dysfunction in the J20 line of human amyloid precursor protein transgenic mice. *J Neurosci* 31(29):10427–10431
- Derecki NC, Katzmarski N, Kipnis J, Meyer-Luehmann M (2014) Microglia as a critical player in both developmental and late-life CNS pathologies. *Acta Neuropathol* 128(3):333–345
- Deutsch LH, Rovner BW (1991) Agitation and other noncognitive abnormalities in Alzheimer's disease. *Psychiatr Clin N Am* 14(2):341–351
- Dhikav V, Anand K (2011) Potential predictors of hippocampal atrophy in Alzheimer's disease. *Drugs Aging* 28(1):1–11
- Dhikav V, Anand KS (2012) Are vascular factors linked to the development of hippocampal atrophy in Alzheimer's disease? *J Alzheimers Dis* 32:711–718
- Do TM, Alata W, Dodacki A, Traversy MT, Chacun H, Pradier L, Scherrmann JM, Farinotti R, Calon F, Bourasset F (2014) Altered cerebral vascular volumes and solute transport at the blood-brain barriers of two transgenic mouse models of Alzheimer's disease. *Neuropharmacology* 81:311–317
- Donkin JJ, Stukas S, Hirsch-Reinshagen V, Namjoshi D, Wilkinson A, May S, Chan J, Fan J, Collins J, Wellington CL (2010) ATP-binding cassette transporter A1 mediates the beneficial effects of the liver X receptor agonist GW3965 on object recognition memory and amyloid burden in amyloid precursor protein/presenilin 1 mice. *J Biol Chem* 285(44):34144–34154
- Duffy AM, Hölscher C (2013) The incretin analogue D-Ala2GIP reduces plaque load, astrogliosis and oxidative stress in an APP/PS1 mouse model of Alzheimer's disease. *Neuroscience* 228:294–300
- Eckman EA, Eckman CB (2005) Abeta-degrading enzymes: modulators of Alzheimer's disease pathogenesis and targets for therapeutic intervention. *Biochem Soc Trans* 33(Pt 5):1101–1105
- El-Amouri SS, Zhu H, Yu J, Marr R, Verma IM, Kindy MS (2008) Nepilysin: an enzyme candidate to slow the progression of Alzheimer's disease. *Am J Pathol* 172(5):1342–1354
- Elcombe EL, Lagopoulos J, Duffy SL, Lewis SJ, Norrie L, Hickie IB, Naismith SL (2015) Hippocampal volume in older adults at risk of cognitive decline: the role of sleep, vascular risk, and depression. *J Alzheimers Dis* 44(4):1279–1290
- Erickson KI, Prakash RS, Voss MW, Chaddock L, Heo S, McLaren M, Pence BD, Martin SA, Vieira VJ, Woods JA, McAuley E, Kramer AF (2010) Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. *J Neurosci* 30(15):5368–5375
- Ferencz B, Gerritsen L (2015) Genetics and underlying pathology of dementia. *Neuropsychol Rev* 25(1):113–124
- Filali M, Lalonde R, Rivest S (2011) Anomalies in social behaviors and exploratory activities in an APPswe/PS1 mouse model of Alzheimer's disease. *Physiol Behav* 104(5):880–885
- Filali M, Lalonde R, Theriault P, Julien C, Calon F, Planel E (2012) Cognitive and non-cognitive behaviors in the triple transgenic mouse model of Alzheimer's disease expressing mutated APP, PS1, and Mapt (3xTg-AD). *Behav Brain Res* 234(2):334–342
- Garcia-Alloza M, Borrelli LA, Hyman BT, Bacskaí BJ (2010) Antioxidants have a rapid and long-lasting effect on neuritic abnormalities in APP:PS1 mice. *Neurobiol Aging* 31(12):2058–2068
- Gengler S, McClean PL, McCurtin R, Gault VA, Hölscher C (2012) Val(8)GLP-1 rescues synaptic plasticity and reduces dense core plaques in APP/PS1 mice. *Neurobiol Aging* 33(2):265–276
- Gil-Bea FJ, Aisa B, Schliebs R, Ramírez MJ (2007) Increase of locomotor activity underlying the behavioral disinhibition in tg2576 mice. *Behav Neurosci* 121(2):340–344
- Guo HB, Cheng YF, Wu JG, Wang CM, Wang HT, Zhang C, Qiu ZK, Xu JP (2015) Donepezil improves learning and memory

- deficits in APP/PS1 mice by inhibition of microglial activation. *Neuroscience* 290:530–542
- Harris JA, Devidze N, Halabisky B, Lo I, Thwin MT, Yu GQ, Breiden DE, Masliah E, Mucke L (2010) Many neuronal and behavioral impairments in transgenic mouse models of Alzheimer's disease are independent of caspase cleavage of the amyloid precursor protein. *J Neurosci* 30(1):372–381
- Holtzman DM, Morris JC, Goate AM (2011) Alzheimer's disease: the challenge of the second century. *Sci Transl Med* 3(77):77sr1
- Hooijmans CR, Graven C, Dederen PJ, Tanila H, van Groen T, Kiliaan AJ (2007) Amyloid beta deposition is related to decreased glucose transporter-1 levels and hippocampal atrophy in brains of aged APP/PS1 mice. *Brain Res* 1181:93–103
- Hsiao YH, Hung HC, Chen SH, Gean PW (2014) Social interaction rescues memory deficit in an animal model of Alzheimer's disease by increasing BDNF-dependent hippocampal neurogenesis. *J Neurosci* 34(49):16207–16219
- Huang H, Wang L, Cao M, Marshall C, Gao J, Xiao N, Hu G, Xiao M (2015) Isolation housing exacerbates Alzheimer's disease-like pathophysiology in aged APP/PS1 mice. *Int J Neuropsychopharmacol* 18:pyu116
- Huang Y, Mucke L (2012) Alzheimer mechanisms and therapeutic strategies. *Cell* 148(6):1204–1222
- Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β . *Sci Transl Med* 4(147):147ra111
- Jankowsky JL, Melnikova T, Fadale DJ, Xu GM, Slunt HH, Gonzales V, Younkin LH, Younkin SG, Borchelt DR, Savonenko AV (2005) Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci* 25(21):5217–5224
- Jankowsky JL, Slunt HH, Ratovitski T, Jenkins NA, Copeland NG, Borchelt DR (2001) Co-expression of multiple transgenes in mouse CNS: a comparison of strategies. *Biomol Eng* 17(6):157–165
- Janus C, Flores AY, Xu G, Borchelt DR (2015) Behavioral abnormalities in APPSwe/PS1dE9 mouse model of AD-like pathology: comparative analysis across multiple behavioral domains. *Neurobiol Aging* 36(9):2519–2532
- Jiang T, Yu JT, Tian Y, Tan L (2013) Epidemiology and etiology of Alzheimer's disease: from genetic to non-genetic factors. *Curr Alzheimer Res* 10(8):852–867
- Ke HC, Huang HJ, Liang KC, Hsieh-Li HM (2011) Selective improvement of cognitive function in adult and aged APP/PS1 transgenic mice by continuous non-shock treadmill exercise. *Brain Res* 1403:1–11
- Kilgore M, Miller CA, Fass DM, Hennig KM, Haggarty SJ, Sweatt JD, Rumbaugh G (2010) Inhibitors of class I histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology* 35(4):870–880
- Kim J, Castellano JM, Jiang H, Basak JM, Parsadanian M, Pham V, Mason SM, Paul SM, Holtzman DM (2009) Overexpression of low-density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular a beta clearance. *Neuron* 64(5):632–644
- Knafo S, Venero C, Merino-Serrais P, Feraud-Espinosa I, Gonzalez-Soriano J, Ferrer I, Santpere G, DeFelipe J (2009) Morphological alterations to neurons of the amygdala and impaired fear conditioning in a transgenic mouse model of Alzheimer's disease. *J Pathol* 219(1):41–51
- Kobayashi K, Hayashi M, Nakano H, Shimazaki M, Sugimori K, Koshino Y (2004) Correlation between astrocyte apoptosis and Alzheimer changes in gray matter lesions in Alzheimer's disease. *J Alzheimers Dis* 6(6):623–632
- Kobayashi K, Hayashi M, Nakano H, Fukutani Y, Sasaki K, Shimazaki M, Koshino Y (2002) Apoptosis of astrocytes with enhanced lysosomal activity and oligodendrocytes in white matter lesions in Alzheimer's disease. *Neuropathol Appl Neurobiol* 28(3):238–251
- Lalonde R, Kim HD, Fukuchi K (2004) Exploratory activity, anxiety, and motor coordination in bigenic APPSwe + PS1/DeltaE9 mice. *Neurosci Lett* 369(2):156–161
- Langbaum JB, Fleisher AS, Chen K, Ayutyanont N, Lopera F, Quiroz YT, Caselli RJ, Tariot PN, Reiman EM (2013) Ushering in the study and treatment of preclinical Alzheimer disease. *Nat Rev Neurol* 9(7):371–381
- Lassalle JM, Halley H, Dumas S, Verret L, Francés B (2008) Effects of the genetic background on cognitive performances of TG2576 mice. *Behav Brain Res* 191(1):104–110
- Li PP, Wang WP, Liu ZH, Xu SF, Lu WW, Wang L, Wang XL (2014) Potassium 2-(1-hydroxypropyl)-benzoate promotes long-term potentiation in A β 1-42-injected rats and APP/PS1 transgenic mice. *Acta Pharmacol Sin* 35(7):869–878
- Lipsky RH, Marini AM (2007) Brain-derived neurotrophic factor in neuronal survival and behavior-related plasticity. *Ann N Y Acad Sci* 1122:130–143
- Liu HL, Zhao G, Cai K, Zhao HH, Shi LD (2011) Treadmill exercise prevents decline in spatial learning and memory in APP/PS1 transgenic mice through improvement of hippocampal long-term potentiation. *Behav Brain Res* 218(2):308–314
- Lo SC, Searce-Levie K, Sheng M (2016) Characterization of social behaviors in caspase-3 deficient mice. *Sci Rep* 6:18335
- Manczak M, Reddy PH (2012)) abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease. *Hum Mol Genet* 21(23):5131–5146
- Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ (2010) Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 330(6012):1774
- Mei X, Ezan P, Giaume C, Koulakoff A (2010) Astroglial connexin immunoreactivity is specifically altered at β -amyloid plaques in β -amyloid precursor protein/presenilin1 mice. *Neuroscience* 171(1):92–105
- Meyer-Luehmann M, Mielke M, Spires-Jones TL, Stoothoff W, Jones P, Bacskai BJ, Hyman BT (2009) A reporter of local dendritic translocation shows plaque-related loss of neural system function in APP-transgenic mice. *J Neurosci* 29(40):12636–12640
- Mhatre SD, Tsai CA, Rubin AJ, James ML, Andreasson KI (2015) Microglial malfunction: the third rail in the development of Alzheimer's disease. *Trends Neurosci* 38(10):621–636
- Minkeviciene R, Ihalaenen J, Malm T, Matilainen O, Keksa-Goldsteine V, Goldsteins G, Iivonen H, Leguit N, Glennon J, Koistinaho J, Banerjee P, Tanila H (2008) Age-related

- decrease in stimulated glutamate release and vesicular glutamate transporters in APP/PS1 transgenic and wild-type mice. *J Neurochem* 105(3):584–594
- Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW (2000) A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408(6815):982–985
- Morishima-Kawashima M (2014) Molecular mechanism of the intramembrane cleavage of the β -carboxyl terminal fragment of amyloidprecursor protein by γ -secretase. *Front Physiol* 5: 463
- Nasrallah IM, Wolk DA (2014) Multimodality imaging of Alzheimer disease and other neurodegenerative dementias. *J Nucl Med* 55(12):2003–2011
- Nelson RL, Guo Z, Halagappa VM, Pearson M, Gray AJ, Matsuoka Y, Brown M, Martin B, Iyuan T, Maudsley S, Clark RF, Mattson MP (2007) Prophylactic treatment with paroxetine ameliorates behavioral deficits and retards the development of amyloid and tau pathologies in 3xTgAD mice. *Exp Neurol* 205(1):166–176
- Obregon D, Hou H, Deng J, Giunta B, Tian J, Darlington D, Shahaduzzaman M, Zhu Y, Mori T, Mattson MP, Tan J (2012) sAPP- α modulates β -secretase activity and amyloid- β generation. *Nat Commun* 3:777
- Oksman M, Iivonen H, Högges E, Amtul Z, Penke B, Leenders I, Broersen L, Lütjohann D, Hartmann T, Tanila H (2006) Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice. *Neurobiol Dis* 23(3): 563–572
- O'Leary TP, Brown RE (2009) Visuo-spatial learning and memory deficits on the Barnes maze in the 16-month-old APPswe/PS1dE9 mouse model of Alzheimer's disease. *Behav Brain Res* 201(1):120–127
- Osborn LM, Kamphuis W, Wadman WJ, Hol EM (2016) Astroglia: an integral player in the pathogenesis of Alzheimer's Disease. *Prog Neurobiol*
- Owen JB, Di Domenico F, Sultana R, Perluigi M, Cini C, Pierce WM, Butterfield DA (2009) Proteomics-determined differences in the concanavalin-A-fractionated proteome of hippocampus and inferior parietal lobule in subjects with Alzheimer's disease and mild cognitive impairment: implications for progression of AD. *J Proteome Res* 8(2):471–482
- Park JH, Widi GA, Gimbel DA, Harel NY, Lee DH, Strittmatter SM (2006) Subcutaneous Nogo receptor removes brain amyloid-beta and improves spatial memory in Alzheimer's transgenic mice. *J Neurosci* 26(51):13279–13286
- Peng S, Garzon DJ, Marchese M, Klein W, Ginsberg SD, Francis BM, Mount HT, Mufson EJ, Salehi A, Fahnestock M (2009) Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease. *J Neurosci* 29(29):9321–9329
- Peng S, Wu J, Mufson EJ, Fahnestock M (2005) Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem* 93(6):1412–1421
- Peng Y, Hu Y, Xu S, Li P, Li J, Lu L, Yang H, Feng N, Wang L, Wang X (2012) L-3-n-butylphthalide reduces tau phosphorylation and improves cognitive deficits in A β PP/PS1-Alzheimer's transgenic mice. *J Alzheimers Dis* 29(2):379–391
- Pereda-Pérez I, Popović N, Ojalora BB, Popović M, Madrid JA, Rol MA, Venero C (2013) Long-term social isolation in the adulthood results in CA1 shrinkage and cognitive impairment. *Neurobiol Learn Mem* 106:31–39
- Perrin RJ, Fagan AM, Holtzman DM (2009) Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature* 461(7266):916–922
- Pistell PJ, Zhu M, Ingram DK (2008) Acquisition of conditioned taste aversion is impaired in the amyloid precursor protein/presenilin 1 mouse model of Alzheimer's disease. *Neuroscience* 152(3):594–600
- Potter R, Patterson BW, Elbert DL, Ovod V, Kasten T, Sigurdson W, Mawuenyega K, Blazey T, Goate A, Chott R, Yarasheski KE, Holtzman DM, Morris JC, Benzinger TL, Bateman RJ (2013) Increased in vivo amyloid- β 42 production, exchange, and loss in presenilin mutation carriers. *Sci Transl Med* 5(189):189ra77
- Qiu WQ, Folstein MF (2006) Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. *Neurobiol Aging* 27(2):190–198
- Querfurth HW, LaFerla FM (2010) Alzheimer's disease. *N Engl J Med* 362(4):329–344
- Sadowski M, Pankiewicz J, Scholtzova H, Ji Y, Quartermain D, Jensen CH, Duff K, Nixon RA, Gruen RJ, Wisniewski T (2004) Amyloid-beta deposition is associated with decreased hippocampal glucose metabolism and spatial memory impairment in APP/PS1 mice. *J Neuropathol Exp Neurol* 63(5):418–428
- Sahlholm K, Liao F, Holtzman DM, Xu J, Mach RH (2015) Sigma-2 receptor binding is decreased in female, but not male, APP/PS1 mice. *Biochem Biophys Res Commun* 460(2):439–445
- Rademakers R, Rovelet-Lecrux A (2009) Recent insights into the molecular genetics of dementia. *Trends Neurosci* 32(8):451–461
- Ramakers IH, Verhey FR, Scheltens P, Hampel H, Soininen H, Aalten P, Rikkert MO, Verbeek MM, Spijru L, Blennow K, Trojanowski JQ, Shaw LM, Visser PJ (2013) Anxiety is related to Alzheimer cerebrospinal fluid markers in subjects with mild cognitive impairment. *Psychol Med* 43(5):911–920
- Rantamäki T, Kempainen S, Autio H, Stavén S, Koivisto H, Kojima M, Anttila H, Miettinen PO, Kärkkäinen E, Karpova N, Vesa L, Lindemann L, Hoener MC, Tanila H, Castrén E (2013) The impact of Bdnf gene deficiency to the memory impairment and brain pathology of APPswe/PS1dE9 mouse model of Alzheimer's disease. *PLoS One* 8(7):e68722
- Reiserer RS, Harrison FE, Syverud DC, McDonald MP (2007) Impaired spatial learning in the APPswe + PSEN1DeltaE9 bigenic mouse model of Alzheimer's disease. *Genes Brain Behav* 6(1):54–65
- Roberts RO, Knopman DS, Przybelski SA, Mielke MM, Kantarci K, Preboske GM, Senjem ML, Pankratz VS, Geda YE, Boeve BF, Ivnik RJ, Rocca WA, Petersen RC, Jack CR Jr (2014) Association of type 2 diabetes with brain atrophy and cognitive impairment. *Neurology* 82(13):1132–1141
- Samaroo HD, Opsahl AC, Schreiber J, O'Neill SM, Marconi M, Qian J, Carvajal-Gonzalez S, Tate B, Milici AJ, Bales KR, Stephenson DT (2012) High throughput object-based image

- analysis of β -amyloid plaques in human and transgenic mouse brain. *J Neurosci Methods* 204(1):179–188
- Schipper HM, Bennett DA, Liberman A, Bienias JL, Schneider JA, Kelly J, Arvanitakis Z (2006) Glial heme oxygenase-1 expression in Alzheimer disease and mild cognitive impairment. *Neurobiol Aging* 27(2):252–261
- Schnabel J (2011) Amyloid: little proteins, big clues. *Nature* 475(7355):S12–S14
- Schröder J, Pantel J (2016) Neuroimaging of hippocampal atrophy in early recognition of Alzheimer's disease—a critical appraisal after two decades of research. *Psychiatry Res* 247:71–78
- Scott L, Feng J, Kiss T, Needle E, Atchison K, Kawabe TT, Milici AJ, Hajós-Korcsok E, Riddell D, Hajós M (2012) Age-dependent disruption in hippocampal θ oscillation in amyloid- β overproducing transgenic mice. *Neurobiol Aging* 33(7):1481.e13–1481.e23
- Shukla V, Zheng YL, Mishra SK, Amin ND, Steiner J, Grant P, Kesavapany S, Pant HC (2013) A truncated peptide from p35, a Cdk5 activator, prevents Alzheimer's disease phenotypes in model mice. *FASEB J* 27(1):174–186
- Smale G, Nichols NR, Brady DR, Finch CE, Horton WE Jr (1995) Evidence for apoptotic cell death in Alzheimer's disease. *Exp Neurol* 133(2):225–230
- Sood A, Warren Beach J, Webster SJ, Terry AV, Buccafusco JJ (2007) The effects of JWB1-84-1 on memory-related task performance by amyloid Abeta transgenic mice and by young and aged monkeys. *Neuropharmacology* 53(5):588–600
- Sudduth TL, Greenstein A, Wilcock DM (2013) Intracranial injection of Gammagard, a human IVIg, modulates the inflammatory response of the brain and lowers A β in APP/PS1 mice along a different time course than anti-A β antibodies. *J Neurosci* 33(23):9684–9692
- Tanzi RE, Bertram L (2008) Alzheimer's disease: the latest suspect. *Nature* 454(7205):706–708
- Tanzi RE, Moir RD, Wagner SL (2004) Clearance of Alzheimer's Abeta peptide: the many roads to perdition. *Neuron* 43(5):605–608
- Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S (2008) New insights into brain BDNF function in normal aging and Alzheimer disease. *Brain Res Rev* 59(1):201–220
- Tarasoff-Conway JM, Carare RO, Osorio RS, Glodzik L, Butler T, Fieremans E, Axel L, Rusinek H, Nicholson C, Zlokovic BV, Frangione B, Blennow K, Ménard J, Zetterberg H, Wisniewski T, de Leon MJ (2015) Clearance systems in the brain—implications for Alzheimer disease. *Nat Rev Neurol* 11(8):457–470
- Trinchese F, Liu S, Battaglia F, Walter S, Mathews PM, Arancio O (2004) Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice. *Ann Neurol* 55(6):801–814
- van de Pol LA, van der Flier WM, Korf ES, Fox NC, Barkhof F, Scheltens P (2007) Baseline predictors of rates of hippocampal atrophy in mild cognitive impairment. *Neurology* 69(15):1491–1497
- van Groen T, Kiliaan AJ, Kadish I (2006) Deposition of mouse amyloid beta in human APP/PS1 double and single AD model transgenic mice. *Neurobiol Dis* 23(3):653–662
- Wang DS, Lipton RB, Katz MJ, Davies P, Buschke H, Kuslansky G, Verghese J, Younkin SG, Eckman C, Dickson DW (2005) Decreased neprilysin immunoreactivity in Alzheimer disease, but not in pathological aging. *J Neuropathol Exp Neurol* 64(5):378–385
- Wang H, He J, Zhang R, Zhu S, Wang J, Kong L, Tan Q, Li XM (2012) Sensorimotor gating and memory deficits in an APP/PS1 double transgenic mouse model of Alzheimer's disease. *Behav Brain Res* 233(1):237–243
- Wang Q, Xu Z, Tang J, Sun J, Gao J, Wu T, Xiao M (2013) Voluntary exercise counteracts A β 25–35-induced memory impairment in mice. *Behav Brain Res* 256:618–625
- Varamini B, Sikalidis AK, Bradford KL (2014) Resveratrol increases cerebral glycogen synthase kinase phosphorylation as well as protein levels of drebrin and transthyretin in mice: an exploratory study. *Int J Food Sci Nutr* 65(1):89–96
- Webster SJ, Bachstetter AD, Nelson PT, Schmitt FA, Van Eldik LJ (2014) Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. *Front Genet* 5:88
- Webster SJ, Bachstetter AD, Van Eldik LJ (2013) Comprehensive behavioral characterization of an APP/PS-1 double knock-in mouse model of Alzheimer's disease. *Alzheimers Res Ther* 5(3):28
- Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, Morgan D (2004) Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation* 1:24
- Wirhlich O, Erck C, Martens H, Harmeier A, Geumann C, Jawhar S, Kumar S, Multhaup G, Walter J, Ingelsson M, Degerman-Gunnarsson M, Kalimo H, Huitinga I, Lannfelt L, Bayer TA (2010) Identification of low molecular weight pyroglutamate a{beta} oligomers in Alzheimer disease: a novel tool for therapy and diagnosis. *J Biol Chem* 285(53):41517–41524
- Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ, Takano T, Deane R, Nedergaard M (2013) Sleep drives metabolite clearance from the adult brain. *Science* 342(6156):373–377
- Xu G, Green CC, Fromholt SE, Borchelt DR (2012) Reduction of low-density lipoprotein receptor-related protein (LRP1) in hippocampal neurons does not proportionately reduce, or otherwise alter, amyloid deposition in APP^{swe}/PS1^{dE9} transgenic mice. *Alzheimers Res Ther* 4(2):12
- Xu Z, Xiao N, Chen Y, Huang H, Marshall C, Gao J, Cai Z, Wu T, Hu G, Xiao M (2015) Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain A β accumulation and memory deficits. *Mol Neurodegener* 10:58
- Xu ZQ, Zhang LQ, Wang Q, Marshall C, Xiao N, Gao JY, Wu T, Ding J, Hu G, Xiao M (2013) Aerobic exercise combined with antioxidative treatment does not counteract moderate- or mid-stage Alzheimer-like pathophysiology of APP/PS1 mice. *CNS Neurosci Ther* 19(10):795–803
- Xu ZQ, Huang H, Chen Y, Gao JY, Xu J, Marshall C, Cai ZY, Xiao M (2016) Different expression patterns of amyloid- β precursor protein secretases in human and mouse hippocampal neurons: a potential contribution to species differences in neuronal susceptibility to amyloid- β pathogenesis. *J Alzheimers Dis* 51(1):179–195
- Yanagisawa D, Ibrahim NF, Taguchi H, Morikawa S, Hirao K, Shirai N, Sogabe T, Tooyama I (2015) Curcumin derivative with the substitution at C-4 position, but not curcumin, is

- effective against amyloid pathology in APP/PS1 mice. *Neurobiol Aging* 36(1):201–210
- Yasojima K, Akiyama H, McGeer EG, McGeer PL (2001) Reduced neprilysin in high plaque areas of Alzheimer brain: a possible relationship to deficient degradation of beta-amyloid peptide. *Neurosci Lett* 297(2):97–100
- Yavuz BB, Ariogul S, Cankurtaran M, Oguz KK, Halil M, Dagli N, Cankurtaran ES (2007) Hippocampal atrophy correlates with the severity of cognitive decline. *Int Psychogeriatr* 19(4):767–777
- Yousefi BH, von Reutem B, Scherübl D, Manook A, Schwaiger M, Grimmer T, Henriksen G, Förster S, Drzezga A, Wester HJ (2015) FIBT versus florbetaben and PiB: a preclinical comparison study with amyloid-PET in transgenic mice. *EJNMMI Res* 5:20
- Zahs KR, Ashe KH (2010) ‘too much good news’—are Alzheimer mouse models trying to tell us how to prevent, not cure, Alzheimer’s disease? *Trends Neurosci* 33(8):381–389
- Zhang Y, Zou J, Yang J, Yao Z (2015) 4A β 1-15-derived monoclonal antibody reduces more A β burdens and neuroinflammation than homologous vaccine in APP/PS1 mice. *Curr Alzheimer Res* 12(4):384–397
- Zhang ZY, Daniels R, Schluesener HJ (2013) Oridonin ameliorates neuropathological changes and behavioural deficits in a mouse model of cerebral amyloidosis. *J Cell Mol Med* 17(12):1566–1576