

# Memantine Leads to Behavioral Improvement and Amyloid Reduction in Alzheimer's-Disease-Model Transgenic Mice Shown as by Micromagnetic Resonance Imaging

Henrieta Scholtzova,<sup>1</sup> Youssef Z. Wadghiri,<sup>2</sup> Moustafa Douadi,<sup>2</sup>  
Einar M. Sigurdsson,<sup>3,4</sup> Yong-Sheng Li,<sup>1</sup> David Quartermain,<sup>1</sup>  
Pradeep Banerjee,<sup>5</sup> and Thomas Wisniewski<sup>1,3,4\*</sup>

<sup>1</sup>Department of Neurology, New York University School of Medicine, New York, New York

<sup>2</sup>Department of Radiology, New York University School of Medicine, New York, New York

<sup>3</sup>Department of Psychiatry, New York University School of Medicine, New York, New York

<sup>4</sup>Department of Pathology, New York University School of Medicine, New York, New York

<sup>5</sup>Forest Research Institute, Jersey City, New Jersey

Memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to improve learning and memory in several preclinical models of Alzheimer's disease (AD). Memantine has also been shown to reduce the levels of amyloid  $\beta$  (A $\beta$ ) peptides in human neuroblastoma cells as well as to inhibit A $\beta$  oligomer-induced synaptic loss. In this study, we assessed whether NMDA receptor inhibition by memantine in transgenic mice expressing human amyloid-beta precursor protein (APP) and presenilin 1 (PS1) is associated with cognitive benefit and amyloid burden reduction by using object recognition, micromagnetic resonance imaging ( $\mu$ MRI), and histology. APP/PS1 Tg mice were treated either with memantine or with vehicle for a period of 4 months starting at 3 months of age. After treatment, the mice were subjected to an object recognition test and analyzed by *ex vivo*  $\mu$ MRI, and histological examination of amyloid burden.  $\mu$ MRI was performed following injection with gadolinium-DTPA-A $\beta$ <sub>1–40</sub>. We found that memantine-treated Tg mice performed the same as wild-type control mice, whereas the performance of vehicle-treated Tg mice was significantly impaired ( $P = 0.0081$ , one-way ANOVA). Compared with vehicle-treated animals, memantine-treated Tg mice had a reduced plaque burden, as determined both histologically and by  $\mu$ MRI. This reduction in amyloid burden correlates with an improvement in cognitive performance. Thus, our findings provide further evidence of the potential role of NMDA receptor antagonists in ameliorating AD-related pathology. In addition, our study shows, for the first time, the utility of  $\mu$ MRI in conjunction with gadolinium-labeled A $\beta$  labeling agents to monitor the therapeutic response to amyloid-reducing agents.  
© 2008 Wiley-Liss, Inc.

**Key words:** amyloid; Alzheimer's disease; NMDA antagonist; micromagnetic resonance imaging

Alzheimer's disease (AD), a chronic neurodegenerative disorder characterized clinically by progressive loss of cognitive and behavioral function, is the most common form of dementia in the world (Sadowski and Wisniewski, 2007). The underlying pathogenesis of AD is caused by neuronal loss related to the abnormal extracellular accumulation of amyloid-beta (A $\beta$ ) peptide in oligomeric form and in neuritic plaques, as well as the intraneuronal aggregation of hyperphosphorylated tau in the form of neurofibrillary tangles (Blennow et al., 2006). There are several other factors that contribute to neuronal degeneration, including inflammation, oxidative stress, and glutamatergic dysfunction.

The pathogenesis of AD is multifactorial. It has been proposed that inappropriate activation of glutamate N-methyl-D-aspartate (NMDA) receptors is responsible for part of the neuronal toxicity and memory and learning impairment observed in AD (Hynd et al., 2004; Chohan and Iqbal, 2006). Abnormalities in glutamatergic signaling associated with AD have been linked to excitotoxicity caused by the excessive influx of Ca through the NMDA receptor calcium channel during sustained

\*Correspondence to: Thomas Wisniewski, MD, New York University School of Medicine, Millhauser Laboratory, Room HN419, 550 First Avenue, New York, NY 10016.  
E-mail: thomas.wisniewski@med.nyu.edu

Received 2 October 2007; Revised 27 December 2007; Accepted 6 February 2008

Published online 9 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.21713

low-level stimulation of glutamatergic neurons (Parsons et al., 1999). Cumulative evidence indicates that glutamate-related alteration in AD can be corrected to some extent by NMDA receptor antagonists such as memantine (Banerjee et al., 2005; Robinson and Keating, 2006).

Memantine efficacy is believed to be related to its low to moderate level of affinity for the NMDA receptor calcium channel, strong voltage dependence, and rapid blocking/unblocking kinetics (Parsons et al., 1999; Robinson and Keating, 2006). Because of these pharmacological characteristics, memantine can preferentially inhibit pathological stimulation of the receptor without disrupting physiological NMDA receptor functioning, which is critical for learning and memory (Parsons et al., 1999). Hence, memantine does not disrupt normal glutamatergic transmission or affect glutamate levels at therapeutically relevant doses (Lipton et al., 2007).

The present study was undertaken to assess whether memantine's role as an NMDA antagonist is associated with therapeutic effects on behavior and amyloid plaque burden in APP/PS1 transgenic mice, by using object recognition and stereological assessments of plaque burden. In addition, we sought to determine whether our previously reported  $\mu$ MRI method for detecting amyloid plaques (Wadghiri et al., 2003; Sigurdsson et al., 2008) could be used to document a therapeutic response of amyloid burden reduction.

## MATERIALS AND METHODS

### Animals and Treatment

The studies were performed in a transgenic mouse model (Tg; APP/PS1-21) with overexpression of mutated amyloid precursor protein (APP; KN670/671NL) and presenilin 1 (PS-1; L166P) under the control of a postnatal, neuron-specific Thy-1 promoter (Radde et al., 2006). The background strain of these mice is C57BL/6J. Progressive cerebral amyloidosis and associated pathology in these animals begin at approximately 6–8 weeks of age. The animals used in this study were maintained on a 12-hr light-dark cycle. All mouse care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the New York University School of Medicine. Two groups (7 mice/group) of 3-month-old APP/PS1 mice were treated either with memantine (10 mg/kg/day; i.p.) or with vehicle (water) for a period of 4 months. At the end of this period, mice were subjected to object recognition testing.

### Behavioral Testing: Novel-Object Recognition Test

The object recognition test is based on the natural tendency of rodents to investigate a novel object instead of a familiar one (Frick and Gresack, 2003). The choice to explore the novel object reflects the use of learning and recognition memory processes. This test does not require food or water deprivation. The object recognition test was conducted in a square open-field box (48 cm square, with 18 cm high walls constructed from black Plexiglas). The light intensity was set

to 30lx. The test consists of a familiarization session (day 1, 15 min) in which mice explored the open-field arena containing two identical, symmetrically placed objects (object A). Mice were trained (day 2, 15 min) with two novel, identically placed objects (object B). Novel-object recognition was tested 3 hr after the training session, when mice were exposed to object B and a novel object C for 6 min. The animals were monitored using an automatic tracking system (San Diego Instruments, San Diego, CA), which records time (in seconds) spent in a zone containing object B or C. The data are presented as percentage differences, calculated by subtracting the percentage time spent by mice exploring the novel object from the percentage time spent exploring the familiar object. The behavioral study was performed in seven memantine-treated Tg animals. Seven age-matched vehicle-treated Tg mice and seven non-Tg age-matched littermates were used as controls.

### Magnetic Resonance Imaging

**Contrast agents and injection.** Magnetically labeled peptides were produced by chelating gadolinium (Gd) to synthetic A $\beta$ <sub>1–40</sub> containing a chelating arm (DTPA) attached to the amino terminus, as previously described (Wadghiri et al., 2003; Sigurdsson et al., 2008). Gd-DTPA-A $\beta$ <sub>1–40</sub> peptide was HPLC purified, lyophilized, and dissolved in water (4 mg/ $\mu$ l). Immediately before administration of the peptide, the solution was mixed with 15% mannitol (w/v in PBS, total volume 600  $\mu$ l) to open the blood-brain barrier (BBB) transiently (Wadghiri et al., 2003; Danysz and Parsons, 2003). The Gd-DTPA-A $\beta$ <sub>1–40</sub> peptide was injected under anesthesia into the right common carotid artery (CCA), using a PHD2000 syringe pump (Harvard Apparatus, Holliston, MA), at a rate of 0.25 ml/kg/sec. These studies were performed in both the memantine-treated and the nonmemantine-treated mice at the age of 7 months, when Tg APP/PS1-21 mice have a substantial A $\beta$  plaque burden (Radde et al., 2006). An additional control group included age-matched wild-type (WT) mice, which also received the Gd-DTPA-A $\beta$ <sub>1–40</sub> peptide in mannitol injection.

**Ex vivo  $\mu$ MRI.** Six hours after intracarotid injection, the mice were anesthetized with sodium pentobarbital (150 mg/kg, i.p.) and perfused transaortically with 0.1 M PBS, pH 7.4, followed by 4% paraformaldehyde in PBS. All experiments were assessed with a 7T SMIS/Magnex system (gradient 250 mT/m, 200  $\mu$ sec rise time). The system was enabled to scan up to four brains at a time by using a litz coil (Doty Scientific; ID = 25 mm, length = 22 mm) and a 30 cc syringe (OD = 24 mm, ID = 20.5 mm); one brain was glued into place in each quadrant of the syringe plunger and immersed in Fomblin (Solvay Solexis Inc., Thorofare, NJ). Fomblin provides a completely dark background on an MRI image, because it does not contain hydrogen protons; hence it was used to provide good, dark contrast around the brains being imaged (Magnitsky et al., 2005). A 3D gradient echo (GE) sequence sensitized to the presence of either iron or Gd-A $\beta$ <sub>1–40</sub> peptide labeling plaques was used to provide 3D T2\*-weighted data sets for plaque visualization [50  $\mu$ m isotropic spatial resolution TR = 50 msec, TE = 5 msec, flip angle

(FA) = 18°, matrix = 512<sup>3</sup>, imaging time = 14 hr 35 min]. The brain data sets were analyzed with the highest detail through virtual resectioning using Analyze software (Lexena, KS). To assess an absolute quantification of the effect of the plaques on the MRI signal, a 2D multigradient echo sequence was acquired as well [four echoes, TR = 1.5 sec, TE = 7.24 msec, echo spacing (ES) = 7.5 msec, FA = 55°, 100 μm × 100 μm × 250 μm, 1 hr]. The corresponding apparent transverse relaxation time T2\* was derived from several brain regions defined by region of interest.

### Histological Studies

After μMRI, the brains were placed in 2% DMSO/20% glycerol in PBS overnight or until sectioning. Serial coronal brain sections (40 μm) were cut and five series of sections at 0.2-mm intervals saved for histological analysis using 1) 6E10/4G8-, 2) thioflavin-S-, 3) GFAP-, and 4) CD45-stained sections, as we have previously described (Sadowski et al., 2006). Aβ deposits were stained with a mixture of monoclonal antibodies 6E10/4G8 or thioflavin-S for fibrillar amyloid. GFAP is a component of the glial intermediate filaments; it forms part of the cytoskeleton and is found predominantly in astrocytes. CD45, a protein-tyrosine phosphatase, is normally moderately expressed on microglia and is a commonly used marker for microglial activation (Sadowski et al., 2006). Both astrocytes and microglia are associated with amyloid deposits. The series were placed in cryoprotectant (30% sucrose/30% ethylene glycol in 0.1 mol/liter phosphate buffer) and stored at -20°C until used. Immunostaining of 6E10/4G8, GFAP, and CD45 was performed as previously described (Sadowski et al., 2006). Briefly, free-floating sections were incubated in 6E10/4G8, both monoclonal anti-Aβ antibodies (Signet, Dedham, MA), at a 1:1,000 dilution for 3 hr. A mouse-on-mouse immunodetection kit (Vector, Burlingame, CA) was used with the biotinylated anti-mouse IgG secondary antibody reacted for 1 hr at a 1:1,000 dilution. Antibody staining was revealed with 3,3'-diaminobenzidine (DAB; Sigma-Aldrich, St. Louis, MO) with nickel ammonium sulfate intensification. GFAP (polyclonal; 1:500; 3 hr; Dako, Glostrup, Denmark) was performed with the primary antibody diluent composed of 2% Triton X-100, 0.1% sodium azide, 0.01% bacitracin, 2% bovine serum albumin, and 10% normal goat serum in PBS, and the secondary biotinylated goat anti-rabbit antibody (Vector) was reacted for 1 hr at 1:1,000 dilution. CD45 (rat anti-mouse; 1:1,000; 3 hr; Serotec, Bicester, United Kingdom) staining was performed similarly to GFAP staining except that the secondary antibody was goat anti-rat (Vector) diluted 1:1,000. Thioflavin-S staining was performed on mounted sections, as published previously (Sadowski et al., 2006).

### Image Analysis

Immunostained tissue sections were quantified with a Bioquant stereology image analysis system (R&M Biometrics Inc., Nashville, TN) using random unbiased sampling, as published previously (Sadowski et al., 2006). All procedures were performed by an individual blinded to the experimental condition of the study. Aβ burden (defined as the percentage of test area occupied by Aβ) was quantified in the neocortex and

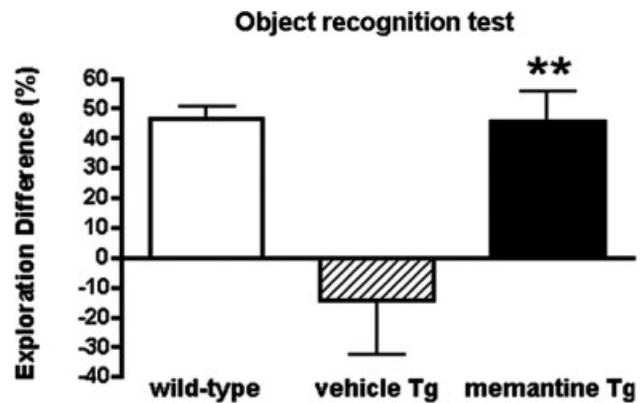


Fig. 1. Memantine significantly improved short-term memory in APP/PS1 mice. At 7 months of age (posttreatment), memantine-treated APP/PS1 mice performed as well as wild-type mice and significantly better than vehicle-treated APP/PS1 mice in a novel-object recognition test (\*\**P* = 0.008 compared with vehicle-APP/PS1 mice; one-way ANOVA). The exploration difference (%) was calculated by subtracting the percentage time spent by mice exploring the novel object from the percentage time spent exploring the familiar object (Bars indicate mean ± standard error of the mean).

in the hippocampus on coronal plane sections stained with the mAb 6E10/4G8. Intensification with nickel ammonium sulfate resulted in black Aβ with minimal background staining that facilitated threshold detection. The cortical area was dorsomedial from the cingulate cortex and extended ventrolaterally to the rhinal fissure within the left hemisphere. Test areas (640 μm × 480 μm) were randomly selected by applying a grid (800 μm × 800 μm) over the traced contour. Hippocampal measurements (600 μm × 600 μm) were performed in a manner similar to the cortical analysis (Sadowski et al., 2006). Quantitative analysis of CD45 microglia and GFAP astrocytes was performed as described above.

### Statistical Analysis

Data from the object recognition test was analyzed by one-way ANOVA (GraphPad Prism 4.0). Differences between groups in Aβ burden, CD45-activated microglia and GFAP astrogliosis within the brain were analyzed by using unpaired two-tailed *t*-tests, whereas the T2\* assessment was analyzed by using a one-tailed *t*-test.

## RESULTS

### Behavioral Studies

After the treatment, mice were subjected to behavioral testing using a novel-object recognition test, which assesses the short-term memory deficits based on an animal's exploratory behavior. Rodents generally exhibit a preference for the novel (or displaced) object, whereas animals with cognitive deficits will not exhibit any exploratory preference. The performance of APP/PS1-21 mice was compared with that of age-matched WT littermates. The treatment with memantine prevented

## Ex vivo $\mu$ MRI

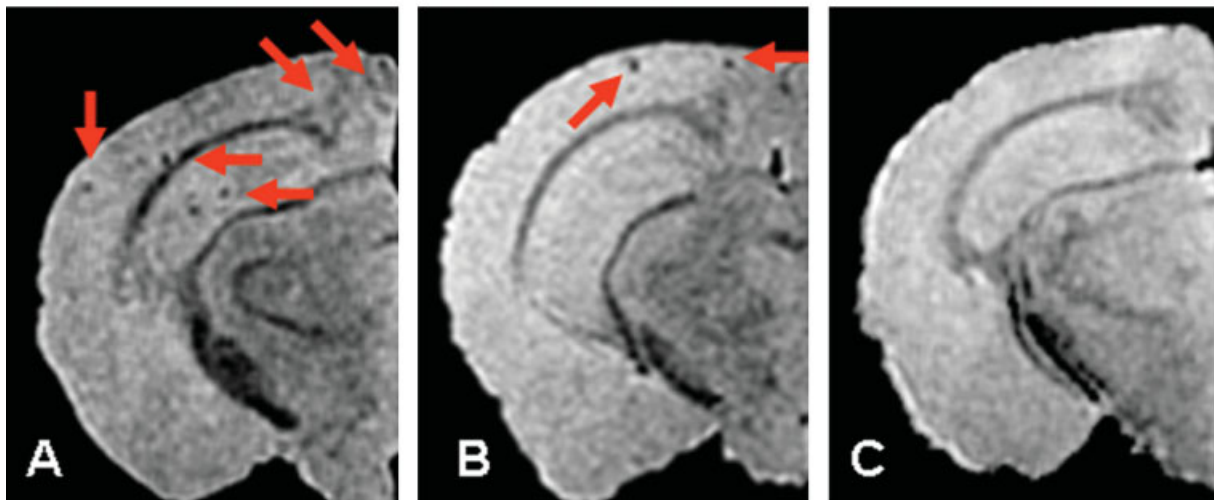


Fig. 2. Memantine decreased amyloid plaque burden in APP/PS1 mice. Micro-MRI scans of vehicle-treated APP/PS1 mice (A), memantine-treated APP/PS1 mice (B), and age-matched wild-type mice (C) at 7 months revealed fewer amyloid plaques (red arrows) in memantine-treated APP/PS1 mice compared with vehicle-treated APP/PS1 mice. A $\beta$  plaques were detected with ex vivo  $\mu$ MRI after intracarotid injection of Gd-DTPA-A $\beta$ <sub>1-40</sub> peptide with manitol.

memory deficits in APP/PS1-2 mice. We found that memantine-treated Tg mice performed similarly to wild-type control mice, whereas the performance of vehicle-treated Tg mice was significantly impaired ( $P = 0.0081$ , one-way ANOVA; Fig. 1).

### MRI Studies

To assess further whether memantine's memory-enhancing effect correlated with its effect on amyloid plaques, the mice were subjected to  $\mu$ MRI image analysis. Our group has previously successfully used gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) to label A $\beta$  peptides to target amyloid plaques in transgenic AD-model mice (Wadghiri et al., 2003, 2005; Sigurdsson et al., 2008). Intracarotid injection of Gd-DTPA-A $\beta$ <sub>1-40</sub>, with mannitol to open the BBB transiently, allowed A $\beta$  plaque detection in the APP/PS1-21 Tg animals.  $\mu$ MRI scans demonstrated a lower amyloid burden in our memantine-treated Tg mice compared with vehicle-treated Tg mice and WT controls (Fig. 2).

Subsequently, T2\* absolute value quantification was performed. The absolute T2\* values were determined in the cortex and hippocampus. A higher T2\* value reflects on average a brighter region with fewer dark spots, which correlates with a lower number of amyloid plaques (Sigurdsson et al., 2008). The cortical T2\* measurements in treated vs. nontreated Tg mice was significantly higher, correlating with the reduced amyloid burden determined histologically ( $P = 0.04$ ,  $t$ -test; Fig. 3).

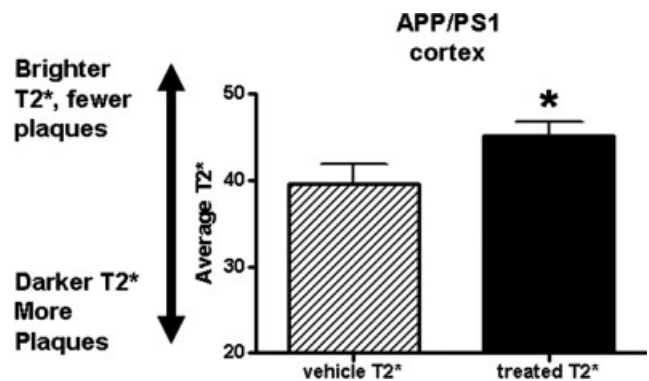


Fig. 3. Average cortical T2\* values in treated vs. control APP/PS1 Tg mice. Quantification of cortical absolute T2\* values also demonstrated a memantine amyloid burden-lowering effect ( $*P = 0.04$ ;  $t$ -test) (Bars indicate mean  $\pm$  standard error of the mean).

### Amyloid Burden and Associated Histopathology

The mice were sacrificed at 7 months of age after 4 months of treatment, and their brains were processed for histology following the  $\mu$ MRI studies, as described elsewhere (Sadowski et al., 2006; Asuni et al., 2006). Histological observation in APP/PS1-21 Tg mice indicated that memantine-treated mice appeared to have fewer plaques compared with vehicle-Tg mice as visualized by thioflavin-S staining and immunostaining (6E10/4G8; Fig. 4).

Quantitative analysis of the amyloid burden, defined as the percentage of area in the measurement

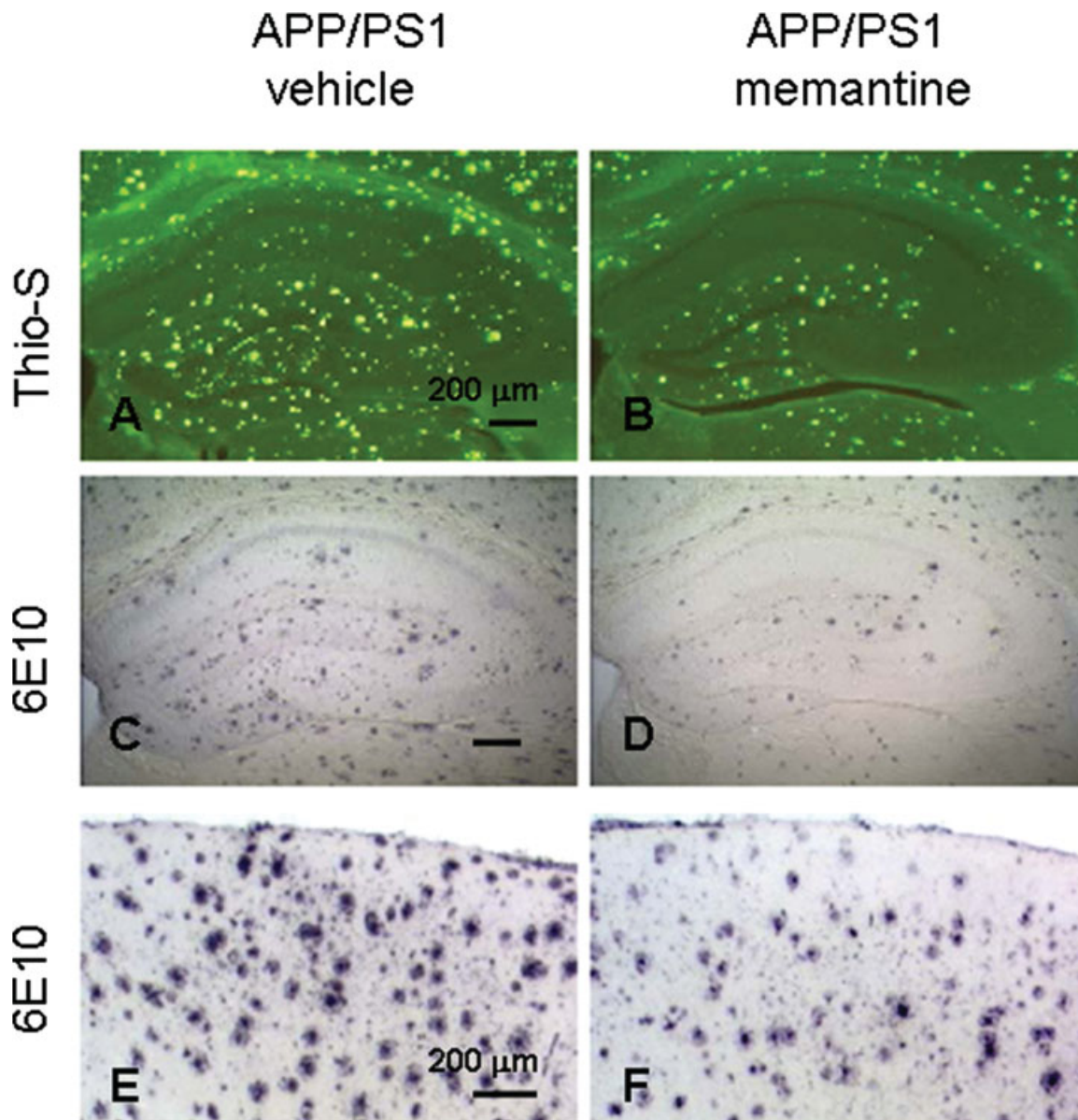


Fig. 4. Memantine decreased hippocampal and cortical plaque burden in APP/PS1 mice. Histological analysis of APP/PS1-21 Tg mice showed the difference in A $\beta$  burden. Thioflavin-S staining revealed more amyloid plaques in hippocampal sections of vehicle-treated APP/PS1 mice (A) compared with memantine-treated APP/PS1 mice (B). Similarly, A $\beta$  immunostaining showed greater A $\beta$  accumu-

lation in hippocampal sections of vehicle-treated APP/PS1 mice (C) compared with sections from memantine-treated APP/PS1 mice (D). Cortical A $\beta$  immunoreactivity also revealed differences between vehicle-treated (E) and memantine-treated (F) APP/PS1 mice. Scale bars = 200  $\mu$ m.

field occupied by reaction product, was determined on the immunostained sections by stereological techniques. After 4 months of memantine administration, the A $\beta$  load in the neocortex and in the hippocampus of treated animals was 25% ( $P = 0.047$ ) and 28% ( $P = 0.021$ ) lower compared with age-matched control Tg animals, which received vehicle (Fig. 5).

In addition to the analysis of A $\beta$  burden in the parenchyma, we evaluated the treatment effect of meman-

tine on inflammatory responses. Subsequent staining for CD45 microglia and for GFAP astrocytes was performed. There was a trend for a reduction of CD45-immunoreactive microglia in memantine-treated Tg animals, but the difference lacked statistical significance (Fig. 6). Quantitative analysis of astroglial staining with GFAP also indicated a trend for reduced astroglial activation in the memantine-treated mice compared with their Tg controls, but the differences were not statistically significant (Fig. 7).

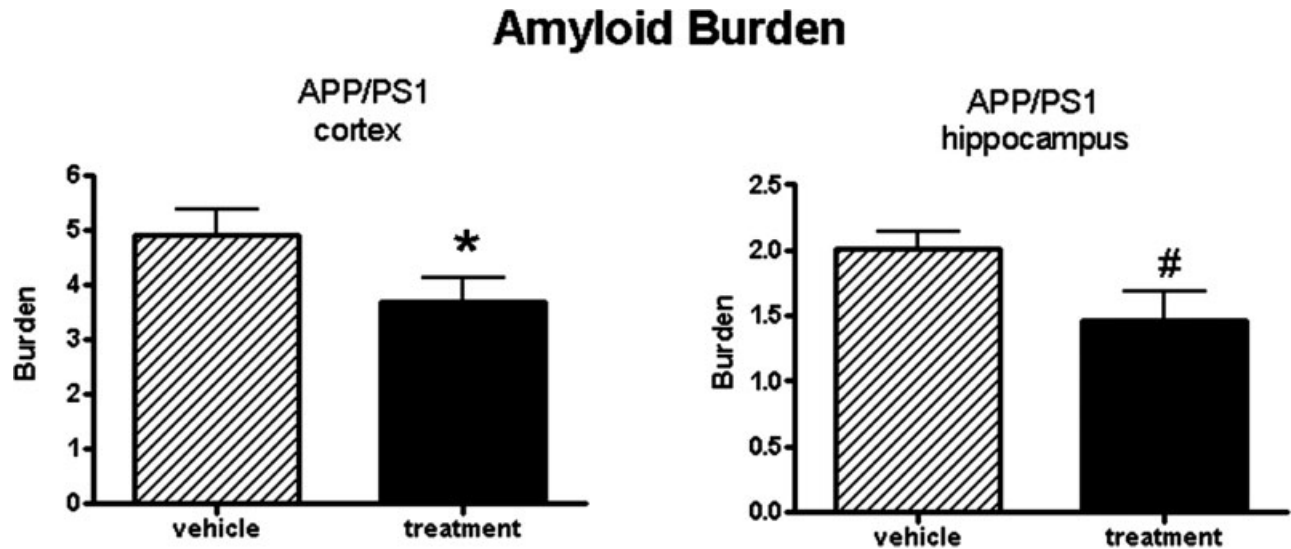


Fig. 5. Memantine reduced amyloid burden in APP/PS1 mice. Significant reduction in the area covered by A $\beta$  (A $\beta$  load) was observed in APP/PS1-Tg mice treated with memantine compared with age-matched Tg control mice treated with vehicle. There was a 25% reduction in cortical amyloid burden (\* $P = 0.047$ , two-tailed t-test) and a 28% reduction in hippocampal amyloid burden ( $^{\#}P = 0.021$ ) as quantified by using an unbiased random sampling scheme and semiautomated image analysis system (Bars indicate mean  $\pm$  standard error of the mean).

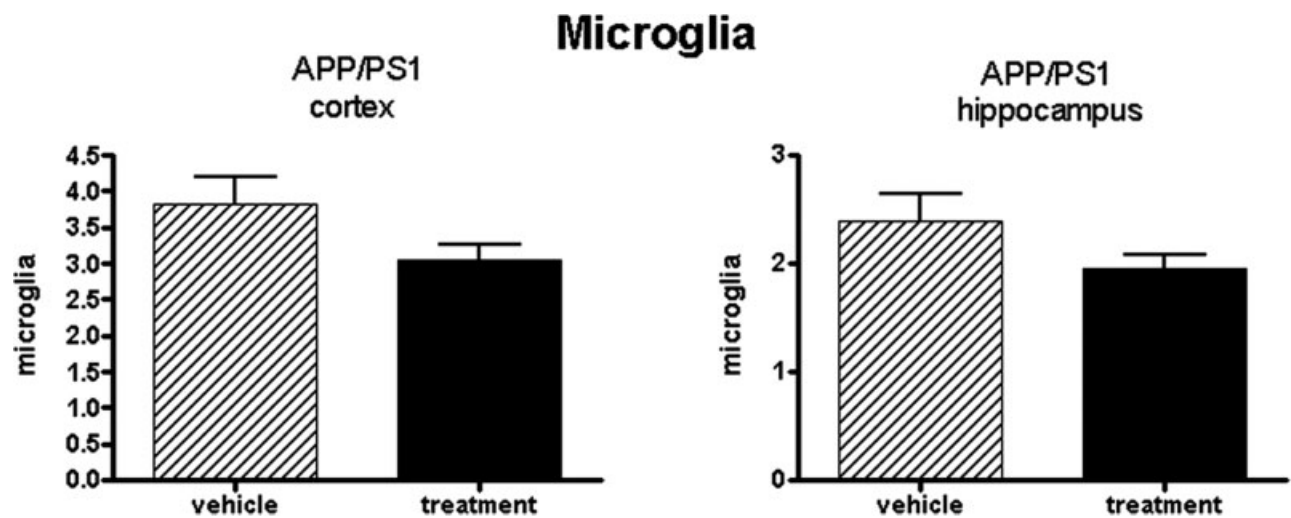


Fig. 6. Memantine reduced cortical and hippocampal CD45 microglia in APP/PS1 mice. CD45 immunostaining followed by stereological analysis revealed fewer activated microglia in memantine-treated Tg animals compared with vehicle-treated animals, but the difference was not statistically significant (Bars indicate mean  $\pm$  standard error of the mean).

## DISCUSSION

One of the major targets of current treatment strategies under development is to lower levels of toxic A $\beta$  species (Wisniewski and Sigurdsson, 2007; Sadowski and Wisniewski, 2007). It is postulated that NMDA receptors are also involved in A $\beta$ -induced neurotoxicity

(Hynd et al., 2004; Banerjee et al., 2005; Chohan and Iqbal, 2006; De Felice et al., 2007). Memantine, a moderate-affinity noncompetitive NMDA receptor antagonist has been shown to improve learning and memory in several preclinical models of AD and is widely used clinically to treat AD (Parsons et al., 1999; Danysz and Par-

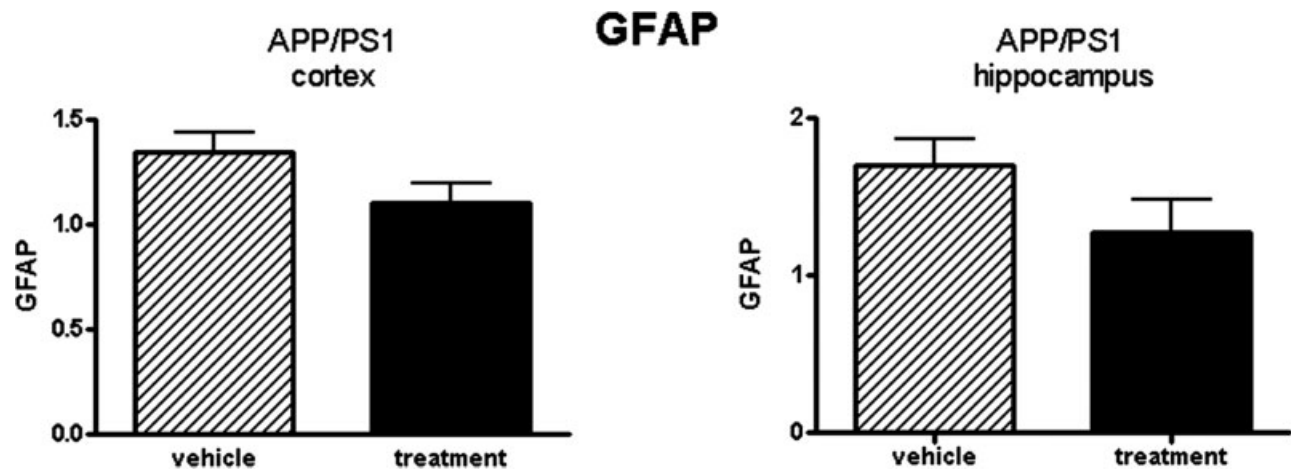


Fig. 7. Memantine reduced cortical and hippocampal GFAP in APP/PS1 mice. GFAP immunostaining followed by stereological analysis revealed fewer activated astrocytes in memantine-treated Tg animals compared with vehicle-treated animals, but the difference was not statistically significant (Bars indicate mean  $\pm$  standard error of the mean).

sons, 2003; Robinson and Keating, 2006). Several studies have suggested a relationship between anomalous glutamatergic activity and the amyloidogenic pathway. For example, it has been observed that chronic NMDAR activation promotes neuronal A $\beta$  production (Lesne et al., 2005). Neurotoxicity produced by A $\beta$  oligomers has been shown to be associated with glutamate excitotoxicity (Lacor et al., 2007; Shankar et al., 2007; De Felice et al., 2007). Earlier reports point to a direct role of memantine in diminishing the production and toxicity of A $\beta$  peptide. For instance, memantine has been shown to reduce the levels of APP, A $\beta$ <sub>1-40</sub>, and A $\beta$ <sub>1-42</sub> peptides in human neuroblastoma cells and in rat primary cortical neurons and to provide neuroprotection against A $\beta$  neurotoxicity (Miguel-Hidalgo et al., 2002; Lahiri et al., 2003a,b). Memantine treatment for as little as 10 days has been shown to reduce the cortical levels of APP in AD Tg mice by 45–55% (Unger et al., 2006). More recent studies using cultured neuronal cells revealed a correlation between the amyloid-reducing effects of memantine and changes in the activity of secretases. In addition, memantine has been found to reduce  $\beta$ -secretase activity (Lahiri et al., 2006). The behavioral treatment effect of memantine seemed to translate into improvements in cognition in various models of impaired memory and learning (Barnes et al., 1996; Parsons et al., 1999; Danysz and Parsons, 2003). Memantine had the ability to ameliorate learning in transgenic AD mice with high brain levels of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> (Minkeviciene et al., 2004) and delayed the decline of cognitive functions in moderate to severe AD patients (Reisberg et al., 2003; Robinson and Keating, 2006). In addition, in AD patients, memantine has been recently shown to reduce cerebrospinal fluid (CSF) levels of phosphorylated tau protein, a relatively specific marker of AD pathology, which has been linked to amyloid deposition-induced neurotoxicity (Degerman et al., 2007).

In this study, we assessed whether memantine's memory-enhancing effects were correlated with its effects on amyloid plaque levels in APP/PS1 transgenic mice using object discrimination and MRI imaging. Transgenic mice expressing human APP and PS1 develop amyloid deposits from the age of about 2 months (Radde et al., 2006). Fourteen APP/PS1 Tg mice (7/group) were treated either with memantine (10 mg/kg; i.p.) or with vehicle for a period of 4 months starting at 3 months of age. After treatment, the mice were subjected to an object discrimination test and analyzed by ex vivo  $\mu$ MRI and histological examination of amyloid burden.  $\mu$ MRI was performed following intracarotid injection with gadolinium-DTPA-A $\beta$ <sub>1-40</sub> (Wadghiri et al., 2003). Coronal brain sections were then stained with thioflavin-S or processed for A $\beta$  immunostaining. We found that memantine-treated Tg mice performed the same as wild-type control mice, whereas the performance of vehicle-treated Tg mice was significantly impaired ( $P = 0.0081$ , one-way ANOVA). Compared with vehicle-treated animals, memantine-treated Tg mice had fewer A $\beta$  plaque lesions, reduced plaque burden, and reduced A $\beta$  immunostaining in the hippocampus and cortex. Memantine treatment in this AD model reduces amyloid burden as assessed by both histological and  $\mu$ MRI studies. The unbiased  $\mu$ MRI studies were able to detect the difference in amyloid burden, even though the difference between the control and the treated groups is relatively modest. This represents the first demonstration that  $\mu$ MRI can be used to follow a therapeutic response in targeting amyloid deposition and opens the possibility of using this method for longitudinal studies. This will allow the evaluation of different types of interventions in a single model animal and permit the more optimal use of nonhuman primates for AD therapeutic drug studies, where histological evaluation

may not be practical. We also showed that the reduction in amyloid burden concurs with an improvement in cognitive performance. Our studies provide further data supporting the hypothesis that NMDA antagonists such as memantine can affect the pathogenesis of AD and potentially provide more than symptomatic relief. Our study also demonstrates, for the first time, the potential of using  $\mu$ MRI in conjunction with gadolinium-labeled ligands to follow therapeutic amyloid-reducing interventions in model animals.

### ACKNOWLEDGMENTS

This work was supported by NIH grants AG15408 and AG20245, as well as by a grant from Forest Research Institute.

### REFERENCES

- Asuni A, Boutajangout A, Scholtzova H, Knudsen E, Li Y, Quartermain D, Frangione B, Wisniewski T, Sigurdsson EM. 2006.  $A\beta$  derivative vaccination in alum adjuvant prevents amyloid deposition and does not cause brain microhemorrhages in Alzheimer's model mice. *Eur J Neurosci* 24:2530–2542.
- Banerjee PK, Lahiri DK, Tanila H, Miguel-Hidalgo JJ, Iqbal K. 2005. Preclinical basis for the efficacy of memantine in Alzheimer's disease. *Biol Psychiatry* 57:173S–174S.
- Barnes CA, Danysz W, Parsons CG. 1996. Effects of the uncompetitive NMDA receptor antagonist memantine on hippocampal long-term potentiation, short-term exploratory modulation and spatial memory in awake, freely moving rats. *Eur J Neurosci* 8:565–571.
- Blennow K, De Leon MJ, Zetterberg H. 2006. Alzheimer's disease. *Lancet* 368:387–403.
- Chohan MO, Iqbal K. 2006. From tau to toxicity: emerging roles of NMDA receptor in Alzheimer's disease. *J Alzheimers Dis* 10:81–87.
- Danysz W, Parsons CG. 2003. The NMDA receptor antagonist memantine as a symptomatological and neuroprotective treatment for Alzheimer's disease: preclinical evidence. *Int J Geriatr Psychiatry* 18:S23–S32.
- De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, Klein WL. 2007. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem* 282:11590–11601.
- Degerman GM, Kilander L, Basun H, Lannfelt L. 2007. Reduction of phosphorylated tau during memantine treatment of Alzheimer's disease. *Dement Geriatr Cogn Disord* 24:247–252.
- Frick KM, Gresack JE. 2003. Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. *Behav Neurosci* 117:1283–1291.
- Hynd MR, Scott HL, Dodd PR. 2004. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochem Int* 45:583–595.
- Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL. 2007. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci* 27:796–807.
- Lahiri DK, Alley GM, Chen DM, Ge YW, Farlow MR, Banerjee PK. 2003a. Effects of memantine on the beta-amyloid precursor protein. *Biol Psychiatry* 53:112S.
- Lahiri DK, Alley GM, Morgan C, Banerjee PK, Farlow MR. 2003b. Effect of memantine on levels of the amyloid beta peptide in cell cultures. *J Neurochem* 85:42.
- Lahiri DK, Chen D, Alley GM, Banerjee PK. 2006. Effects of memantine on the activity of secretase enzymes in human neuroblastoma cells. *Eur Neuropsychopharmacol* 16:S483–S484.
- Lesne S, Ali C, Gabriel C, Croci N, MacKenzie ET, Glabe CG, Plotkine M, Marchand-Verrecchia C, Vivien D, Buisson A. 2005. NMDA receptor activation inhibits alpha-secretase and promotes neuronal amyloid-beta production. *J Neurosci* 25:9367–9377.
- Lipton SA. 2007. Pathologically-activated therapeutics for neuroprotection: mechanism of NMDA receptor block by memantine and S-nitrosylation. *Curr Drug Targets* 8:621–632.
- Lipton SA, Gu Z, Nakamura T. 2007. Inflammatory mediators leading to protein misfolding and uncompetitive/fast off-rate drug therapy for neurodegenerative disorders. *Int Rev Neurobiol* 82:1–27.
- Magnitsky S, Watson DJ, Walton RM, Pickup S, Bulte JW, Wolfe JH, Poptani H. 2005. In vivo and ex vivo MRI detection of localized and disseminated neural stem cell grafts in the mouse brain. *Neuroimage* 26:744–754.
- Miguel-Hidalgo JJ, Alvarez XA, Cacabelos R, Quack G. 2002. Neuroprotection by memantine against neurodegeneration induced by beta-amyloid(1–40). *Brain Res* 958:210–221.
- Minkeviciene R, Banerjee P, Tanila H. 2004. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. *J Pharmacol Exp Ther* 311:677–682.
- Parsons CG, Danysz W, Quack G. 1999. Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist—a review of preclinical data. *Neuropharmacology* 38:735–767.
- Radde R, Bolmont T, Kaeser S, Coomaraswamy J, Lindau D, Stoltze L, Calhoun ME, Jaggi F, Wolburg H, Gengler S, Haas C, Ghetti B, Czech C, Holscher C, Mathews PM, Jucker M. 2006.  $A\beta$ 42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep* 7:940–946.
- Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ. 2003. Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med* 348:1333–1341.
- Robinson DM, Keating GM. 2006. Memantine: a review of its use in Alzheimer's disease. *Drugs* 66:1515–1534.
- Sadowski M, Wisniewski T. 2007. Disease modifying approaches for Alzheimer's pathology. *Curr Pharmaceutical Design* 13:1943–1954.
- Sadowski M, Pankiewicz J, Scholtzova H, Mehta P, Prelli F, Quartermain D, Wisniewski T. 2006. Blocking the apolipoproteinE/amyloid  $\beta$  interaction reduces the parenchymal and vascular amyloid- $\beta$  deposition and prevents memory deficit in AD transgenic mice. *Proc Natl Acad Sci U S A* 103:18787–18792.
- Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. 2007. Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J Neurosci* 27:2866–2875.
- Sigurdsson E, Wadghiri YZ, Mosconi L, Blind JA, Knudsen E, Asuni A, Tsui WH, Sadowski M, Turnbull D, de Leon M, Wisniewski T. 2008. A non-toxic ligand for voxel-based MRI analysis of plaques in AD transgenic mice. *Neurobiol Aging*. 29:836–847.
- Unger C, Svedberg MM, Yu WF, Hedberg MM, Nordberg A. 2006. Effect of subchronic treatment of memantine, galantamine, and nicotine in the brain of Tg2576 (APP<sup>swe</sup>) transgenic mice. *J Pharmacol Exp Ther* 317:30–36.
- Wadghiri YZ, Sigurdsson EM, Sadowski M, Elliot JI, Li Q, Scholtzova H, Tang CY, Aguinaldo JG, Pappolla M, Duff K, Turnbull D, Wisniewski T. 2003. Detection of Alzheimer's amyloid in transgenic mice using magnetic resonance microimaging. *Magn Reson Med* 50:293–302.
- Wadghiri YZ, Sigurdsson EM, Wisniewski T, Turnbull D. 2005. MR imaging of amyloid plaques in transgenic mice. In: Sigurdsson EM, editor. *Amyloid proteins: methods and protocols*. Totowa, NJ: Humana Press Inc. p 365–379.
- Wisniewski T, Sigurdsson EM. 2007. Therapeutic approaches for prion and Alzheimer's diseases. *FEBS J* 274:3784–3798.