Published in final edited form as:

Exp Neurol. 2007 May ; 205(1): 166–176.

Prophylactic Treatment with Paroxetine Ameliorates Behavioral Deficits and Retards the Development of Amyloid and Tau Pathologies in 3xTgAD Mice

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Abstract

A history of depression is a risk factor for Alzheimer's disease (AD), suggesting the possibility that antidepressants administered prophylactically might retard the disease process and preserve cognitive function. Here we report that pre-symptomatic treatment with the antidepressant paroxetine attenuates the disease process and improves cognitive performance in the 3xTgAD mouse model of AD. Five month-old male and female 3xTgAD and non-transgenic mice were administered either paroxetine or saline daily for 5 months. Open-field activity was tested in 7 month-old mice and performance in passive avoidance and Morris swim tasks were evaluated at 10 months. 3xTgAD mice exhibited reduced exploratory activity, increased transfer latency in the passive avoidance test and impaired performance in the Morris spatial navigation task compared to nontransgenic control mice. Paroxetine treatment ameliorated the spatial navigation deficit in 3xTgAD male and female mice, without affecting swim speed or distance traveled, suggesting a preservation of cognitive function. Levels of amyloid beta-peptide (A β) and numbers of A β immunoreactive neurons were significantly reduced in the hippocampus of male and female paroxetine-treated 3xTgAD mice compared to saline-treated 3xTgAD mice. Female 3xTgAD mice exhibited significantly less tau pathology in the hippocampus and amygdala compared to male 3xTgAD mice, and paroxetine lessened tau pathology in male 3xTgAD mice. The ability of a safe and effective antidepressant to suppress neuropathological changes and improve cognitive performance in a mouse model, suggests that such drugs administered prophylactically might retard the development of AD in humans.

Introduction

Alzheimer's disease (AD) is characterized by progressive impairment of memory accompanied by psychiatric disturbances (Lyketsos et al., 2002; Petersen, 2004). Behavioral abnormalities in AD result from dysfunction and death of neurons in brain regions involved in cognition and mood such as the hippocampus, amygdala and associated cortical regions. These brain regions suffer from degeneration of synapses and neurons, which are associated with extracellular accumulations of amyloid β -peptide (A β) (plaques) and intraneuronal filamentous aggregates

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of the microtubule-associated protein tau (neurofibrillary tangles) (Selkoe and Schenk, 2003;Mattson, 2004). A β is a 40–42 amino acid proteolytic cleavage product of an integral membrane protein, the amyloid precursor protein (APP). Molecular genetic analyses of subjects with early-onset inherited familial AD (FAD) identified mutations in APP, and presenilins 1 and 2 as the causes of some cases of FAD; in each case the mutation increases the production of the long form of A β (A β 1–42) (De Strooper, 2003;Hardy, 2004). A β and tau pathologies are closely associated in ways that suggest a role for A β in the formation of neurofibrillary tangles (Giasson et al., 2003), although the number of neurofibrillary tangles is more highly correlated with the severity of cognitive impairment among AD patients than is A β pathology (Bierer et al., 1995). Moreover, neurofibrillary tangles are sufficient, in the absence of A β pathology, to cause dementia as occurs in patients with fronto-temporal lobe dementia with Parkinsonism linked to chromosome 17 (FTDP-17) caused by tau mutations (Ingram and Spillantini, 2002). Transgenic mice that express a FAD APP mutation alone or in combination with a FAD presenilin-1 mutation exhibit progressive A β deposition, synaptic dysfunction and cognitive deficits, but no neurofibrillary tau pathology (Ashe, 2001;Dodart et al., 2002;Cleary et al., 2005). Mice expressing FAD APP and presenilin-1 mutations together with a FTDP-17 tau mutation (3xTgAD mice) exhibit age-dependent A β deposition and tau pathology in the hippocampus, which is associated with impaired synaptic plasticity (Oddo et al., 2003a) and deficits in spatial learning tasks (Billings et al., 2004).

Neurochemical abnormalities in the brains of AD patients include reduced levels of the neurotransmitters acetylcholine, serotonin and norepinephrine (Gottfries, 1990;Meltzer et al., 1998;Gsell et al., 2004). A history of depression is a risk factor for AD (Ownby et al., 2006), and other findings suggest that reduced activation of serotonin signaling pathways might promote synaptic dysfunction and neuronal death in AD (reviewed by Mattson et al., 2004). For example, serotonin may enhance synaptic plasticity by activating cyclic AMP response element-binding protein (CREB) and up-regulating the expression of brain-derived neurotrophic factor (BDNF) (reviewed by Pang and Lu, 2004), and this signaling mechanism may be compromised in AD (Tong et al., 2001). Serotonin-selective reuptake inhibitors are widely prescribed for the treatment of clinical depression and anxiety disorders (Wagstaff et al., 2002;Bourin, 2003), but may also have therapeutic potential as neuroprotective agents (Sanchez et al., 2001;Duan et al., 2004). It was recently reported that environmental enrichment (Lazarov et al., 2004) and dietary restriction (Patel et al., 2005;Wang et al., 2005) can reduce A β deposition in APP mutant mice. Because the antidepressant paroxetine can activate signaling pathways similar to those activated by exercise, cognitive stimulation and dietary restriction (Mattson et al., 2004), we determined whether it might ameliorate AD-like pathology and cognitive impairment in a mouse model.

Materials and Methods

Mice and Treatments.

The generation of homozygous 3xTgAD mice, and characterization of amyloid and tau pathologies and synaptic dysfunction in this line of mice have been described previously (Oddo et al., 2003a; Billings et al., 2004). Experiments were performed using 5 month-old 3xTgAD mice (10 males and 10 females) and nontransgenic control mice (10 males and 10 females). The mice were maintained under a 12 h light/12 h dark cycle with continuous access to food and water. The mice were housed in a colony that had been exposed to mouse hepatitis virus, but were not shedding virus at the time of experimentation. Five male and 5 female mice of each genotype received an intraperitoneal (ip) injection of 5 mg/kg body weight paroxetine (SmithKline Beecham) daily, while the other 5 male and 5 female mice of each genotype received an ip injection of saline each day. Paroxetine was dissolved in saline immediately prior to injection each day. The mice were 5 months old at the time the treatments were initiated

and were treated for a total of 5 months. At 7 months of age, all mice were subjected to an open-field activity test. At 10 months of age all mice were tested in a Morris water maze (MWM), followed 2 days later by a passive avoidance trial. Treatments were continued for 1 week following the behavioral testing and the mice were euthanized 4 h after the last paroxetine or saline injection. Brains were rapidly removed and cut at the midline in the sagittal plane. One hemi-brain was immersion fixed in 4% paraformaldehyde in PBS, and the hippocampus and cortex were dissected from the remaining hemi-brain and immediately frozen and stored at -80°C . All procedures were approved by the Animal Care and Use Committee of the National Institute on Aging.

Behavioral Analyses.

Open field activity was assessed 2 months after initiation of treatments with paroxetine or saline. The test apparatus consisted of a closed square arena (47 cm) equipped with infrared light-sensitive photocells, placed in a quiet dimly lit testing room. Each mouse was placed in the apparatus and ambulatory counts were recorded for a 10 min period. Values were expressed as number of beam crossings/10 min. The Morris spatial navigation task was performed in mice that had been treated with vehicle or paroxetine for 5 months (10 month-old mice). The apparatus consisted of a white circular plastic pool (100 cm diameter and 50 cm high) which was filled with water ($22 \pm 1^{\circ}\text{C}$) to a depth of 30 cm; the water was rendered opaque by addition of milk powder. Spatial navigation cues were affixed to a plexiglass cylinder which was concentric with the pool and extended approximately 60 cm above the top of the pool. A circular (10 cm diameter) white escape platform (the target) was submerged 1 cm below the water surface, 30 cm from the edge of the pool in a position designated as quadrant 2. A camera was mounted on the ceiling in the center of the pool. A Videomex tracking system was used to track the swim route of the mouse and data was collected online using Videomex Water Maze Software (Columbus Instruments, OH) at a sampling rate of 30 samples/second. Each acquisition trial (4 trials per day for 7 days) was started by placing a mouse in the water facing the wall of the tank; the location of entry of the mouse into the water was chosen randomly for each trial. A trial lasted until the mouse found the platform or until 60 s had elapsed. If a mouse did not find the platform within 60 s, it was placed on the platform for 10 s in order to familiarize it with the location of the platform. After the completion of fourth trial on each day, the mouse was returned to its home cage. The passive avoidance apparatus consisted of equal-sized light and dark compartments with a light bulb fixed in the center of the roof of the light compartment. The floor consisted of a metal grid connected to a shock scrambler. The two compartments were separated by a trap door. On the day of testing, the mouse was placed in the light compartment and the time taken to enter the dark compartment was recorded and termed as initial latency (IL). Immediately after the mouse entered the dark chamber the trap door was closed and an electric foot shock (0.1 mA) was delivered for 3 s. Mice that had an initial latency of more than 60 s were excluded from experiments. Twenty four h after the acquisition trial, a second (retention) trial was conducted and the time the mouse took to enter the dark compartment was designated retention latency (RL; recorded to a maximum of 600 s).

ELISA Analysis of A β Levels

Hippocampal and neocortical tissues were homogenized in a buffer containing 100 mM Pipes, 500 mM NaCl, 0.2% Triton X-100, 0.1% NaN_3 , 2% BSA, 0.5mM sodium vanadate, 2 mM EDTA, 200 μM PMSF and a cocktail of protease inhibitors (10.4 mM AEBSF, 8 μM aprotinin, 0.2 mM leupeptin, 0.4mM bestatin, 0.15mM pepstatin A and 0.14 mM E-64. Samples were homogenized on ice at a power level of 4 and pulses at 1 s intervals for 30 s. Samples were allowed to sit on ice for 1 h, centrifuged at $12,000 \times g$ for 15 min (4°C) and supernatants were collected and used for ELISA analyses. Protein concentrations were determined using a BCA kit (Pierce). The concentrations of A β 1–40 and A β 1–42 in the samples was measured by

ELISA using antibodies that selectively recognize full-length A β 1–40 or A β 1–42 as described previously (Horikoshi et al., 2004).

Immunohistochemistry.

Frozen cryoprotected hemi-brains were cut at 30–40 μ m in the coronal plane through the entire rostrocaudal extent of the brain using a freezing microtome and were stored in tissue storage solution (FD Neurotechnologies, Baltimore MD) at -20°C . For tau immunostaining, free floating sections were washed in PBS, incubated for 1 h in 0.5% hydrochloric acid and then washed in PBS. Sections were incubated in 3% H_2O_2 for 30 min, washed with PBS and immunostained using a MOM kit (Vector Labs, Burlingame, CA) according to manufacturer's instructions. The antibody against human tau (antibody HT7; Innogenetics) was used at a dilution 1:200. For visualization of tau immunoreactivity, sections were incubated for 5 min in the presence of diaminobenzidine and nickel, washed with water and mounted onto slides. Sections were dried overnight and then coverslipped. For A β immunostaining, 30 μ m sections were treated as above with modifications. After the H_2O_2 incubation, sections were incubated for 1 h in PBS containing 0.2% Triton X-100 and 5% horse serum. Sections were then incubated in the presence of 6E10 primary antibody (Chemicon) overnight at 4°C . Sections were washed in PBS, incubated in secondary anti-mouse biotinylated antibody (Vector Labs) for 1 h and then washed with PBS. Sections were incubated in the presence of streptavidin-peroxidase complex for 30 min, washed with PBS and then incubated with diaminobenzidine for 1 min.

Statistical Analyses.

Comparisons among the different treatment groups and among different genotypes were made using ANOVA, and multiple pairwise comparisons were made using the Scheffe post-hoc test (SigmaStat).

Results

Paroxetine treatment ameliorates behavioral abnormalities in 3xTgAD mice

We chose a dose of paroxetine (5 mg/kg, i.p.) that had previously been shown to inhibit serotonin reuptake and ameliorate behavioral deficits in mouse models of anxiety and depression (Cryan et al., 2004; Goeldner et al., 2005). Five month-old male and 3xTgAD and non-transgenic control mice were treated daily with either 5 mg/kg paroxetine or saline for a period of 5 months, during which time body weights were recorded weekly. Mice in all groups gained body weight with no significant differences between paroxetine- and saline-treated mice (data not shown). Analyses of open field activity revealed that the spontaneous exploratory activity of 3xTgAD mice (both males and females) was significantly lower than the activity of non-transgenic mice (Fig. 1A). Paroxetine treatment had no significant effect on open field activity in 3xTgAD or nontransgenic mice. The passive avoidance test is based on the preference of the mouse for a dark environment when placed in an adjacent brightly lit area. In the acquisition trial all mice entered the darkened box within 60 s and then received a footshock. In the second session (retention), performed 24 h after the acquisition session, the transfer latency for 3xTgAD mice was significantly greater than the transfer latency of non-transgenic mice (Fig. 1B). Paroxetine treatment had no significant effect on transfer latency in male or female 3xTgAD mice, but resulted in a small and significant increase in transfer latency in non-transgenic mice (Fig. 1B).

The Morris spatial navigation task is widely used to evaluate spatial learning and memory ability in rats and mice (Brandeis et al., 1989; Holmes et al., 2002). After having been treated for 5 months with either paroxetine or saline, 3xTgAD and non-transgenic mice were tested in the spatial navigation task daily (4 trials per day) for 7 days. All mice learned the task as indicated by a reduction in goal latency times over the 7 day training period (Fig. 2A). Saline-

treated non-transgenic mice outperformed saline-treated 3xTgAD mice as indicated by a significantly greater decrease in goal latency over the course of the 7 days of testing. In both male and female 3xTgAD mice paroxetine treatment resulted in a significantly greater decrease in goal latency compared to saline-treated 3xTgAD mice (Fig. 2A). In contrast, paroxetine worsened acquisition in non-transgenic mice. Swim speeds and distance traveled were not significantly different in 3xTgAD mice and nontransgenic mice, and were not significantly affected by paroxetine in 3xTgAD or nontransgenic mice (data not shown), suggesting that the effects of genotype and paroxetine were not the result of differences in motivation or sensory/motor function.

Retention of the memory for the location of the platform was tested in a probe trial in which the platform was removed and the amount of time the mouse spent in the quadrant of the pool where the platform had been located was determined during a 60 s trial. All groups of mice spent more time in the target quadrant than in any of the other quadrants, and there were no significant differences in the amount of time spent in the target quadrant among genotypes or treatment groups (Fig. 2B). Thus, paroxetine had a significant effect on goal latency, without affecting performance in the probe trial, suggesting that paroxetine improved memory acquisition without affecting memory retention.

Amyloid β -Peptide Pathology is Reduced in Paroxetine-Treated 3xTgAD Mice

Previous studies have documented a progressive increase in the amount of A β immunoreactivity in the hippocampus and cerebral cortex of 3xTgAD mice (Oddo et al., 2003a;2003b). To determine whether paroxetine treatment affected the accumulation of A β in these mice we first measured levels of A β 1–40 and A β 1–42 in samples of cerebral cortex of 3xTgAD mice using a sensitive ELISA method (Horikoshi et al., 2004). The A β 1–40 concentration in saline-treated 3xTgAD mice was nearly 3-fold greater in females as compared to males (Fig. 3A). The A β 1–40 concentration in the cerebral cortex samples from paroxetine-treated 3xTgAD mice was significantly lower than the concentration in saline-treated 3xTgAD mice; in both males and females paroxetine treatment reduced A β 1–40 levels by more than 50% (Fig. 3A). In contrast to its effect on A β 1–40 levels, paroxetine treatment had no significant effect on levels of A β 1–42 in the 3xTgAD mice (data not shown). Levels of A β 1–40 and A β 1–42 were below the limit of detection in cerebral cortex samples from non-transgenic mice (data not shown).

We next evaluated A β immunoreactivity in brain sections from saline-treated and paroxetine-treated 3xTgAD mice using antibody 6E10 which recognizes amino acids 1–17 of human A β (Kim et al., 1990). Consistent with the results of the A β ELISA measurements, the intensity of A β immunoreactivity in the hippocampus (Fig. 3B) and cerebral cortex (data not shown) appeared lower in paroxetine-treated mice compared to vehicle-treated mice. Many CA1 hippocampal pyramidal neurons exhibited intracellular A β immunoreactivity in saline-treated 3xTgAD mice, with significantly fewer such neurons with A β accumulations being present in the hippocampi of paroxetine-treated male and female mice (Fig. 3C). Examination of other brain regions revealed the amygdala as another site with A β immunoreactive neurons. Numbers of A β immunoreactive neurons in the amygdala of paroxetine-treated mice were not significantly different than in vehicle-treated mice, in both males and females (Fig. 3D). Plaque-like deposits of A β were not observed at this middle stage of disease progression in the 3xTgAD mice (see Oddo et al., 2003b). No A β immunoreactivity was observed in brain sections from non-transgenic mice (data not shown).

Tau Pathology is Attenuated in Paroxetine-Treated 3xTgAD Male Mice

Previous studies have documented a progressive tau pathology in CA1 pyramidal neurons in the hippocampus of 3xTgAD mice which may be caused by A β accumulation (Oddo et al.,

2003a;2003b). To determine whether paroxetine treatment affects the development of tau pathology in these mice we immunostained brain sections with a tau antibody (HT7) that is specific for human tau (Mercken et al., 1992). Many CA1 hippocampal pyramidal neurons exhibited robust accumulations of tau immunoreactivity in saline-treated male 3xTgAD mice, with fewer such tau-immunoreactive neurons being present in the CA1 neurons of paroxetine-treated male 3xTgAD mice (Fig. 4A). The results of cell counts revealed a highly significant reduction in the numbers of tau immunoreactive CA1 neurons in paroxetine-treated male 3xTgAD mice compared to vehicle-treated 3xTgAD male mice (Fig. 4B). Female 3xTgAD mice exhibited significantly fewer tau immunoreactive CA1 neurons compared to male mice, and paroxetine had no significant effect on the number of tau positive CA1 neurons in the female mice. The amygdala of 3xTgAD mice contained tau immunoreactive neurons (Fig. 4C). The results of cell counts revealed a highly significant reduction in the numbers of tau immunoreactive neurons in the amygdala of paroxetine-treated male 3xTgAD mice compared to vehicle-treated male 3xTgAD mice (Fig. 4D). Female 3xTgAD mice exhibited significantly fewer tau immunoreactive neurons in the amygdala compared to male mice, and paroxetine had no significant effect on the number of tau positive amygdalar neurons in the female mice. No tau immunoreactivity was observed in brain sections from non-transgenic mice (data not shown).

Discussion

Deficits in performance in tests of learning and memory have been documented in studies of several transgenic lines of APP mutant and APP/presenilin-1 double-mutant mice (Hsiao et al., 1996;Morgan et al., 2000;Gordon et al., 2001;Kotilinek et al., 2002). These deficits typically become evident prior to the overt deposition of A β and then intensify as A β pathology progresses. It was recently reported that 3xTgAD mice exhibit impaired performance in learning and memory tasks which are evident as early as 6 months of age (Billings et al., 2005). Indeed, we found that 10 month-old 3xTgAD mice were significantly impaired in the goal latency task, suggesting a deficit in memory acquisition. The improved functional outcome was correlated with reduced levels of A β 1–40 in paroxetine-treated 3xTgAD mice suggesting that paroxetine intervenes in the pathogenic process upstream of A β production. It is thought that small oligomers of A β are particularly damaging to synapses (Lambert et al., 1998;Lacor et al., 2004), possibly by causing membrane-associated oxidative stress which perturbs calcium homeostasis and impairs energy metabolism (Mattson et al., 1992; Keller et al., 1997).

Our analyses were performed on 3xTgAD mice that were at a stage of the disease process in which tissue levels of A β were increased and A β immunoreactivity was accumulated in some neuronal populations (CA1 and amygdalar neurons), but no large aggregates of A β (i.e., plaque-like deposits) were evident. The latter observations are consistent with a role for intracellular and/or extracellular A β oligomers in promoting synaptic dysfunction. Interestingly, while lowering A β 1–40 levels, paroxetine treatment did not decrease A β 1–42 levels. Because A β 1–40 preferentially accumulates in cerebral blood vessels, whereas A β 1–42 accumulates in the brain parenchyma (reviewed by Herzig et al., 2006), our findings suggest a possible suppressive effect of paroxetine on vascular amyloid pathology, although further studies would be required to elucidate this possibility.

Studies of human subjects have established strong correlations between A β levels and neurofibrillary tangles and cognitive deficits (Nagy et al., 1995;Naslund et al., 2000). However, a cause-effect relationship between A β deposition, and tau pathology and synaptic dysfunction, may not apply to all cases of AD, and certainly does not apply to patients with frontotemporal lobe dementia who exhibit abundant tau pathology, but no A β pathology (Froelich-Fabre et al., 2004). In our study, paroxetine treatment lessened the amount of A β in the hippocampus in both males and females. Paroxetine treatment reduced tau pathology in CA1 neurons

significantly; however, CA1 tau pathology in females was less than in males and paroxetine had no effect on CA1 tau pathology in females. Our findings therefore suggest that A β accumulation is not directly related to tau pathology in the 3xTgAD mice. While our data do not allow any firm conclusions as to the relative contributions of A β and tau pathologies to learning and memory impairment in 3xTgAD mice, they do suggest that these pathologies and cognitive dysfunction are amenable to therapeutic intervention.

Mice overexpressing wild-type human APP develop diffuse A β deposits and an age-related deficit in goal latency in the water maze, but no abnormalities in the open field and passive avoidance tests (Koistinaho et al., 2001). In our study, 3xTgAD mice exhibited reduced movement in the open field activity test, suggesting a heightened state of anxiety. However, paroxetine did not affect performance in the open field activity test, nor did paroxetine affect the swim speed or path length of 3xTgAD mice in the water maze, suggesting that the beneficial effect of paroxetine on water maze performance in 3xTgAD mice was likely due to an effect on cognition rather than an anxiolytic action. Interestingly, we found that paroxetine worsened performance of nontransgenic mice, which might be explained by a reduction in anxiety and, hence, motivation to escape from the water. Consistent with this, it has been reported that the anxiolytic action of fluoxetine in mice is associated with impaired performance in learning and memory tasks that involve an aversive stimulus. However, more direct effects of paroxetine on learning and memory are possible. For example, anticholinergic effects of paroxetine have been reported (Fujishiro et al., 2002). If paroxetine impairs cholinergic signaling, which would be expected to worsen performance in tests of spatial memory, such as the water maze, which involve activation of acetylcholine receptors in the hippocampus (Gold, 2003)..

AD patients exhibit heightened anxiety and associated behavioral problems including agitation and depression. The 3xTgAD mice exhibited reduced exploratory behavior in the open field test, suggesting an increased anxiety level compared to nontransgenic mice. The increased transfer latency in the passive avoidance task in 3xTgAD mice is a result expected from impaired memory for the punishment, but could also result from increased anxiety. In contrast to our results with 3xTgAD mice, APP mutant mice (King et al., 1999; Lim et al., 2001) exhibited increased open field activity. This difference may be the result of differential A β pathology in the two different models, or to the tau pathology present in the 3xTgAD mice, but lacking in the APP mutant mice. We found that, in addition to the hippocampus, the amygdala is a site of A β accumulation and tau pathology in 3xTgAD mice. The amygdala, a brain region involved in anxiety behaviors, is a site of both A β and tau pathology in AD patients (Goedert et al., 1991). The amygdalar pathology in the 3xTgAD mice may therefore play a role in their enhanced anxiety-like behavior.

The mechanism by which paroxetine suppresses A β and tau pathologies in 3xTgAD mice remains to be determined. The antidepressant action of paroxetine is believed to be mediated by inhibition of serotonin reuptake resulting in enhanced serotonergic signaling and up-regulation of the expression of BDNF (Russo-Neustadt, 2003; Mattson et al., 2004). Levels of serotonin (Gottfries, 1990; Gsell et al., 2004) and BDNF (Murray et al., 1994; Hock et al., 2000; Lee et al., 2005) are decreased in the hippocampus and cerebral cortex of AD patients. Enhanced serotonergic signaling and/or BDNF signaling might therefore mediate the beneficial effects of paroxetine on A β and tau pathologies and behavior in 3xTgAD mice. However, we found that BDNF levels in the hippocampus were not different in saline- and paroxetine-treated 3xTgAD mice (Supplemental Fig. 1). Because we were only able to measure BDNF levels at one time point at the end of the study after the mice had been subjected to water maze training, it is possible that basal levels of BDNF might have been elevated during the time period prior to behavioral testing. On the other hand, paroxetine treatment may intervene in the AD process independently of its effects on serotonin and BDNF signaling. For example, a recent study demonstrated that paroxetine can reduce A β levels by suppressing

translation of the APP protein (Morse et al., 2004; Tucker et al., 2005), and action that could have contributed to the decreased levels of A β immunoreactivity in the hippocampus of paroxetine-treated 3xTgAD mice.

Previous studies of APP and APP/presenilin-1 double-mutant mice have consistently documented higher amounts of soluble A β and extracellular A β plaques in females compared to males (Wang et al., 2003; Maynard et al., 2006; Pacheco-Quinto et al., 2006). Epidemiological studies indicate that AD is more common in females than in males, and the underlying mechanism for this sex difference has been proposed to involve the higher levels of estrogen in females (Manthey et al., 2001; Zhang et al., 2005). Another explanation may be that because males express more testosterone this leads to reduction in levels of A β since it has been demonstrated that testosterone reduces A β production (Gouras et al., 2000). Interestingly, we found that male 3xTgAD mice exhibited many more tau immunoreactive CA1 and amygdalar neurons compared to females. This occurred despite greater amounts of soluble A β 1–40 and similar numbers of A β immunoreactive neurons in females. Because the available data suggest a causal role for early A β accumulation in the subsequent tau pathology in AD patients (Hardy and Allsop, 1991; Cummings, 2003; Mattson, 2004) and 3xTgAD mice (Oddo et al., 2003b; 2006), our findings suggest the neurons in females may be more resistant to A β toxicity and associated tau pathology compared to neurons in male mice. The basis for this increased resistance of neurons in females to amyloid pathology is not known. We found that paroxetine treatment resulted in approximately a 50% reduction in A β 1–40 levels in both male and female 3xTgAD mice. Paroxetine was also highly effective in reducing tau pathology in male 3xTgAD mice. In light of the excellent safety record of paroxetine in long-term treatment of patients with depression and anxiety disorders, including elderly subjects, our findings suggest that clinical trials of paroxetine in human subjects with mild cognitive impairment and early AD are warranted.

Acknowledgements:

This research was supported by the National Institute on Aging Intramural Research Program, and by a grant to Y. M. (AG022455).

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Supplementary Material

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A

Figure 1.

3xTgAD mice exhibit abnormalities in open field activity and passive avoidance tests, which are not ameliorated by paroxetine. A. Open field activities of male and female nontransgenic control mice and 3xTgAD mice. Values are the mean and SD ($n = 5-10$ mice). $*p < 0.01$ compared to the corresponding value for nontransgenic mice. B. Transfer latency values in the passive avoidance test. Values are the mean and SD ($n = 10$). $*p < 0.05$ compared to the corresponding value for nontransgenic mice.

B **Figure 2.**

Paroxetine treatment ameliorates the impaired performance of 3xTgAD mice in the Morris spatial navigation task. A. Goal latencies (data for males and females combined). Values are the means and SD ($n = 10$). The performance of paroxetine-treated 3xTgAD mice was significantly different than that of vehicle-treated 3xTgAD mice ($p < 0.01$). The performance of paroxetine-treated nontransgenic mice was significantly different than that of vehicle-treated nontransgenic mice ($p < 0.01$). B. Results of probe trial (values for males and females combined). Values are the means and SD ($n = 10$).

A

B

Male

Female

Saline

Paroxetine

C

D

Figure 3.

Aβ accumulation is reduced in paroxetine-treated 3xTgAD mice. A. Aβ₁₋₄₀ levels in the hippocampus of 3xTgAD mice that had been treated with saline or paroxetine. Values are the mean and SD (n = 5). *p<0.05 compared to the corresponding value for vehicle-treated mice. B. Micrographs showing Aβ immunoreactivity in the hippocampus and amygdala of 3xTgAD mice that had been treated with saline or paroxetine. C and D. Results of quantitative analysis of Aβ-positive neurons in region CA1 of the hippocampus (C) and the amygdala (D) of 3xTgAD mice that had been treated with saline or paroxetine. Values are the mean and SD (n = 5).

A

Male

Female

Saline

Paroxetine

B

D **Figure 4.**

Paroxetine treatment attenuates tau pathology in male 3xTgAD mice. A. Micrographs showing tau immunoreactivity in the hippocampus of male and female 3xTgAD mice that had been treated with saline or paroxetine. B. Results of quantitative analysis of tau-positive neurons in region CA1 of the hippocampus of 3xTgAD mice that had been treated with saline or paroxetine. Values are the mean and SD (n = 5). *p<0.05, **p<0.01 compared to the saline value. #p<0.05 compared to the male saline value. C. Micrographs showing tau immunoreactivity in the amygdala of 3xTgAD mice that had been treated with saline or paroxetine. D. Results of quantitative analysis of tau-positive neurons in the amygdala of the hippocampus of 3xTgAD mice that had been treated with saline or paroxetine. Values are the mean and SD (n = 5). *p<0.05 compared to the saline value. #p<0.05 compared to the male saline value.