# The impaired performance of female APP/PS1 mice in the Morris water maze is coupled with increased Aβ accumulation and microglial activation.

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**Running title:** Female APP/PS1 mice display spatial learning impairment and greater Aβ burden

**Keywords:** Alzheimer's disease, spatial memory, neuroinflammation, BACE-1, IDE.

**Abstract**

**Background:** Alzheimer's Disease is characterized by progressive neuronal loss and cognitive decline. Epidemiological studies suggest that the risk of AD is higher in women even when data are adjusted for age.

**Objective:** We set out to compare changes in 9 month-old male and female mice which overexpress amyloid precursor protein (APP) with presenilin (PS1; APP/PS1 mice) and to evaluate whether any changes were coupled with deficits in spatial learning.

**Methods:** APP/PS1 mice were assessed for their ability to learn in the Morris water maze and Aβ burden assessed by Congo Red and Aβ triple ultra-sensitive assay. Neuroinflammatory changes were examined in brain tissue along with expression of Aβ-generating and -degrading enzymes.

**Results:** A deficit in reversal phase learning in the Morris water maze was observed in female mice and was paralleled by evidence of increased accumulation of Aβ, microglial activation and expression of IL-1β. Accumulation of Aβ was coupled with an increase in expression of BACE-1 and a decrease in insulin-degrading enzyme (IDE).

**Conclusion:** The results indicate that the observed impairment in spatial memory in female APP/PS1 mice correlated with increased Aβ burden and the changes in Aβ may have occurred as a result of enhanced BACE-1 and decreased IDE expression.

## Introduction

Alzheimer's disease is a progressive neurodegenerative disorder characterized by two pathological hallmarks – extracellular plaques composed of amyloid-β (Aβ) protein and neurofibrillary tangles composed of hyperphosphorylated tau. A common feature in the brain of AD patients is the presence of astrocytes and microglia surrounding the senile amyloid plaques, and quantitative analysis of the microglial marker, carbon-11-labelled (R)-PK11195, using positron emission topography, has suggested that microglial activation occurs at very early clinical stages of the disease [1]. Indeed there is considerable evidence implicating neuroinflammation in the pathogenesis of AD. Initial support for a role for inflammation in AD arose from epidemiological studies that observed that patients treated with non-steroidal anti-inflammatory drugs exhibited decreased incidence of AD [2] and several more recent reports have provided evidence to support this [3, 4]. Evidence that microglia play a role in inflammation in the CNS of AD patients also came from studies showing that IL-1β, IL-6, and TNF-α expression were upregulated in these individuals, and that they were predominantly associated with AD plaques [5-7]. Interestingly, studies have also linked factors associated with inflammation such as TNF, IL-3, IL-1 and IL-11 to development of AD in MCI and presymptomatic AD patients [8]

Microglia are capable of Aβ phagocytosis as demonstrated by several *in vitro* reports [9-11]. However, recent multiphoton *in vivo* data confirming the persistence of amyloid plaques following formation indicate that microglia do not clear plaques, although some level of phagocytosis may occur on the perimeter of plaques. In animal models of AD, a consistent feature of microglia proximal to amyloid plaques is persistent activation of microglia, characterized by production of proinflammatory molecules, combined with ineffective phagocytosis of Aβ [12].

In AD, accumulation of Aβ can theoretically occur because of insufficient clearance of Aβ. Interestingly phagocytosis by monocytes prepared from patients with AD is reduced [12, 13] and macrophages prepared from post mortem brain tissue also exhibited reduced phagocytic function [12]. However, increased production of Aβ from amyloid precursor protein (APP) and/or decreased degradation are also key factors in modulating Aβ accumulation. Production of Aβ relies on the activity of β-secretase 1 (BACE-1), a transmembrane aspartic protease that cleaves APP whereas several enzymes are capable of degrading Aβ peptides including neprilysin and insulin-degrading enzyme [14]. Neprilysin is a membrane-bound zinc-dependent metalloprotease that degrades a number of peptides including Aβ both *in vivo* [15]and *in vitro* [16-19]. Genetic ablation of neprilysin causes elevation of endogenous Aβ [20] while transgenic expression of neprilysin lowers cerebral Aβ levels [15, 21-24]. Insulin-degrading enzyme (IDE) is a zinc-metalloprotease that shows no sequence specificity for Aβ however it is thought to recognise a conformation that is prone to conversion to β-sheet structure [25]. Similar to neprilysin,

genetic deletion of insulin-degrading enzyme in mice leads to elevated levels of cerebral Aβ [26] whereas its overexpression causes a decrease in brain Aβ levels [23].

There have been conflicting reports in the literature regarding the activity of BACE-1, neprilysin and IDE in animal models of AD with some groups reporting age- and sex-related changes while others reported no changes. Indeed where changes were reported they occurred at much older ages than those of the mice assessed in this study. Here we set out to investigate whether Aβ accumulation was coupled with microglial activation and changes in cognitive function, and to investigate the causes for the sex-related increase in plaque load in female APP/PS1 mice. The data indicate that microglial activation was increased, particularly around Aβ-containing plaques in brain sections prepared from APP/PS1 transgenic mice and that microglial activation was more apparent in female transgenic mice which also displayed greater plaque burden than their male counterparts. Interestingly BACE-1 expression was increased in tissue prepared from female compared with male mice whereas expression of IDE was decreased in tissue prepared from female APP/PS1 mice but not female wildtype mice.

**Methods**

*Animals*

APPswe/PSEN1de9 (Jackson Laboratories, Maine, USA) and litter-mate control male and female mice (n = 7-11) were bred in an SPF animal housing facility in the Bioresources Unit, Trinity College Dublin and maintained under veterinary supervision in a controlled environment (12-hour light-dark cycle; 22-23°C). We did not control for the oestrus cycle in female mice. Animal experimentation was performed under a license granted by the Minister for Health and Children (Ireland) under the Cruelty to Animals Act 1876 and the European Community Directive, 86/609/EEC, and in accordance with local ethical guidelines.

Mean body weights (± SD) at 8-9 months of age were 35.8 ± 5.0, 35.5 ± 6.1, 36.7 ± 5.1 and 41.7 ± 4.8 in female wildtype and APP/PS1 mice and in male wildtype and APP/PS1 mice respectively; the mean value in the male APP/PS1 mice was significantly greater than that of the other groups ($p < 0.05$; ANOVA).

Animals were assessed for muscle strength and coordination using the hangwire and inverted screen tests and the data indicate that the performance of mice in each group was similar at 8 – 9 months of age (data not shown).

*Behavioural analysis*

Behavioural analysis in the Morris water maze took place in a well-lit room (22-23°C) with distinct visual cues placed at a height of 1 metre on the 4 walls surrounding the tank. The diameter and height of the water maze were 1.2m and 0.6m respectively and the depth of the water was 0.24m. The platform diameter was 0.15m and it was placed in the northwest quadrant of the pool, 0.13 m from the edge. Training commenced after 1 day of habitation and continued for 5 consecutive days and each mouse underwent 4 one-minute trials with an inter-trial interval of 5 minutes. Each trial ended when mice located the platform, or after 60 seconds, after which time unsuccessful mice were led to the platform; animals remained on the platform for 20 seconds. Following the training period, the position of the submerged platform was moved diagonally and the animals were assessed in this configuration (reversal phase) daily for 4 days.

*mRNA analysis*

Brain tissue harvested for mRNA analysis was unilaterally dissected at the level of the hippocampus and included cortical and hippocampal tissue. RNA was isolated using Nucleospin® RNAII KIT (Macherey-Nagel, Duren, Germany) and cDNA was prepared using High-Capacity cDNA RT kit according to the manufacturer's instructions (Applied Biosystems, UK). Real-time PCR for the detection of *CD68*, *CD11b*, *GFAP*, *IL-1β*, *BACE-1*, *NEP* and *IDE* mRNA was performed with predesigned Taqman gene expression assays (Applied

Biosystems, UK). mRNA was normalized to the endogenous control, *actin* RNA. Samples were assayed on an Applied Biosystems 7500 Fast Real-Time PCR machine.

*Immunohistochemistry*

Congo red staining of Aβ fibrils was used to identify the presence of plaques in brain tissues from APP/PS1 and wildtype mice. Briefly, sections were fixed in ice-cold ethanol and incubated in saturated NaCl solution for 20 mins at RT. NaOH solution was added to freshly prepared and filtered Congo Red solution and sections incubated in this solution for 30 mins at RT. Sections were rinsed in $ddH_2O$ and counter-stained with methyl green. Plaque number was assessed in 4 coronal brain sections containing cortex and hippocampus from each animal.

*Detection of SDS-soluble and insoluble Aβ by multi-spot ELISA*

Soluble and insoluble Aβ were extracted from snap-frozen brain tissue containing both hippocampal and cortical tissue. Tissue was homogenised in 5 volumes (w/v) buffer (50mM NaCl, 1% SDS (w/v), pH10) containing phosphatase and protease inhibitor cocktails (Sigma, UK) and centrifuged at 15,000rpm for 40 mins at 4°C. SDS-soluble Aβ was assessed in supernatant and insoluble Aβ isolated from the pellets using 5M guanidine. Aβ concentrations were assessed using 96-well multi-spot 4G8 Aβ triple ultra-sensitive assay kits (MesoScale Discovery, USA). Plates were read using a sector Imager plate reader and concentration of Aβ in the samples determined with reference to the standard curve prepared using recombinant Aβ.

*Statistical analysis*

Data were analysed as appropriate using either Student *t*-test for independent means or 2-way analysis of variance (ANOVA) followed by Bonferroni tests to determine which conditions were significantly different from each other. Data are expressed as means ± SEM.

**Results**

*Female APP/PS1 mice exhibit deficits in the reversal phase of a spatial learning task*

Animals were evaluated for their performance in the Morris Water maze and all animals performed similarly in the training phase of the task so that mean latency (Fig 1A) and pathlength on the final day of training were similar in male wildtype and APP/PS1 (2.75 + 0.98; n=7 and 3.27 + 1.89; n=5; respectively) and female wildtype and APP/PS1 (2.13 + 0.94; n=11 and 2.38 + 1.11; n=5; respectively) mice. During the reversal phase of the task (Fig 1B), all animals learned the modified task so that mean latency and pathlength decreased during the 4 days in all treatment groups. However, genotype was a significant factor in the performance of female mice only (latency, $p<0.001$; F(1,21.06); 2-way ANOVA) and so female APP/PS1 mice exhibited a significantly poorer performance on day 4 ($^{**}p<0.01$; Bonferroni post-hoc test). This difference could not be attributed to a difference in average swim speeds which were similar in male and female wildtype mice (0.10 ± 0.01 and 0.11 ± 0.02 mice; mean ± SEM; m/sec) and male and female APP/PS1 mice (0.10 ± 0.02 and 0.10 ± 0.01) mice. No differences were observed between the groups during a reversal probe trial performed 24 h after the end of the reversal phase.

*CD68, CD11b and GFAP mRNA expression are enhanced in brain tissue from APP/PS1 mice.*

Analysis of mRNA expression of both CD68 and CD11b (Fig 2A and B respectively) revealed enhanced expression in brain tissue prepared from female, compared with male, mice ($^{+}p<0.047$; F(1,4.729; $^{+}p<0.0175$; F(1,6.476) respectively; 2-way ANOVA; n=4) and a genotype-related increase in mRNA expression of both of these proteins in male and female mice ($^{*}p<0.0001$; F(1,28.78; $^{*}p<0.0101$; F(1,7.739) respectively; 2-way ANOVA; n=4). Conversely, GFAP mRNA (Fig 2C) expression was significantly decreased in tissue prepared from female, compared with male, mice ($^{+}p<0.0048$; F(1,13.02); 2-way ANOVA; n=4) while a genotype-related increase was found in both male and female mice ($^{*}p<0.0001$; F(1,54.09); 2-way ANOVA; n=4). Inflammatory cytokines are produced by activated glia, particularly microglia, and here we report that IL-1β mRNA expression (Fig 2D) was increased in tissue prepared from female, compared with male, mice ($^{+}p<0.0041$; F(1,15.82); 2-way ANOVA; n=4) and in male and female APP/PS1, compared with wildtype, mice ($^{*}p<0.0001$; F(1,52.36); 2-way ANOVA; n=4).

*Aβ burden is greater in female APP/PS1 mice.*

Since expression of markers of glial activation was different in female, compared with male, mice, Aβ load was investigated in brain tissue from both as Aβ is known to modulate activity of glia in the CNS. To examine plaque load brain, sections were stained with Congo Red (Fig 3A) and a multi-spot ELISA was

used to determine concentrations of soluble and insoluble Aβ (Fig 3C and D) since both species have been shown to alter glial activity [27].

Examination of plaque number using Congo Red immunohistochemistry identified a significantly greater number of Aβ plaques in brain sections prepared from female APP/PS1, compared with male APP/PS1, mice in both cortex and hippocampus (Fig 3B; $^{*}p<0.05$; Student's *t*-test for Independent means). Furthermore, assessment of Aβ concentrations in brain tissue from APP/PS1 mice identified significantly more insoluble $A\beta_{1-42}$, though not soluble $A\beta_{1-42}$, in brain tissue prepared from female APP/PS1, compared with male APP/PS1, mice (Fig 3D; $^{+}p<0.05$; ANOVA; n=4). While APP/PS1 mice displayed higher concentrations of $A\beta_{1-40}$ and $A\beta_{1-38}$ in brain tissue than wildtype mice, no differences in concentration of either of these species were observed in female, compared with male, APP/PS1 mice (data not shown).

*Aβ generation and clearance enzymes are altered in female mice.*

In an effort to understand the mechanism by which female APP/PS1 display greater Aβ burden, the expression of neprilysin, insulin-degrading enzyme and BACE-1 was examined in brain tissue from APP/PS1 mice. There was a significant increase in BACE-1 mRNA expression in brain tissue prepared from female, compared with male, mice (Fig 4C; $^{*}p<0.0077$; F(1,13.64); 2-way ANOVA; n=4) although no genotype-related changes were identified.

In contrast to the production of Aβ through cleavage of APP, neprilysin and insulin-degrading enzyme degrade Aβ peptides [16-20, 23, 26]. Expression of neprilysin mRNA and IDE mRNA was similar in brain tissue from male and female mice, and whereas there was no genotype-related change in neprilysin mRNA (Fig 4A), there was a significant decrease in IDE mRNA expression in tissue prepared from female APP/PS1 mice compared with female wildtype mice (Fig 4B; $^{*}p<0.0162$; F(1,9.215); 2-way ANOVA; n=4) and a sex x genotype interaction in female APP/PS1 mice (Fig 4B; $^{*}p<0.0258$; (F1,7.454; 2-way ANOVA; n=4).

**Discussion**

This study set out to establish whether the changes in plaque deposition in APP/PS1 mice were associated with changes in microglial activation and spatial learning, and to evaluate the impact of sex on these changes. We observed a clear sex-related difference in pathology, with increased plaque numbers and increased insoluble $A\beta_{1-42}$, associated with increased expression of BACE-1 and decreased expression of IDE, in the brain of females; these changes were paralleled by deficits in the reversal phase of the Morris water maze task and microglial activation.

Epidemiological studies suggest that the risk of AD is higher in women even when data are adjusted for age [28, 29] and have linked this with the loss of the neuroprotective effect of oestrogens [30]. However a recent analysis suggested that the issue of increased susceptibility of women needs to be further evaluated [31]. Higher Aβ concentrations have been reported in the brain of 16 month-old, but not 9 month-old, female mice that overexpress APP, PS1 and Tau, compared with their male counterparts [32]. These findings were observed in 16 month-old, but not 9 month-old, mice though other studies using APP/PS1 mice reported that the age-related increase in Aβ in hippocampal was more profound in female compared with male mice and that significant differences were observed particularly in 12 and 17 month-old mice [33]. The impact of the oestrus cycle on the changes in Aβ concentration was not assessed in these studies and remains to be clarified. It has been suggested that differences in brain concentrations of metals may contribute to Aβ deposition. However, while the concentration of manganese is higher in tissue prepared from female transgenic mice, and the concentration of copper is lower, no correlation between Aβ accumulation and metal concentrations has been observed [34].

One of the most significant findings of the present study is that there are marked differences in Aβ concentration and plaque deposition in female, compared with male, APP/PS1 mice as early as 9 months of age. The data suggest that this is a consequence of a sex-related increase in BACE-1 that increases the production of Aβ and decreased IDE which contribute to the clearance of Aβ. The increase in BACE-1 in female, compared with male, APP/PS1 mice described here broadly concurs with other evidence which demonstrated that expression of soluble APPβ, the amino-terminal product of BACE-1 cleavage of APP, was increased in 9 month-old female triple transgenic (3xTg) mice (ie before evidence of a change in Aβ load) [32]. Additionally, the change in BACE-1 expression was apparent in female wildtype mice indicating that the upregulation was not due to Aβ burden. A similar observation has been made in 24-month old C57 mice where BACE-1 activity was enhanced in female, compared with male, mice [35]. Interestingly, Schafer and colleagues [36] reported an age-related decline in BACE-1 activity in 14 week-old male $APP_{751}$ mice therefore it is possible that the increase in BACE-1 observed here in female mice at 9 months is evident because of an age-related decline in BACE-1 in their male counterparts. The impact

of the oestrus cycle on the changes in Aβ concentration or BACE-1 was not assessed in these studies and remains to be clarified.

Clearly, degradation of Aβ also impacts on accumulation of Aβ and several enzymes including neprilysin and IDE are Aβ-degrading enzymes. We report that expression of IDE mRNA was decreased in female transgenic mice compared with their male counterparts, and combined with the increase in BACE-1 expression, may provide an explanation for the increased Aβ load. Interestingly, both IDE mRNA and protein are reduced in hippocampus of AD brains [37] while decreased expression of neprilysin is apparent in areas of the AD brain where plaque burden is greatest [38]. In contrast with the change in IDE expression, we observed no genotype- or sex-related change in neprilysin expression in 9 month-old APP/PS1 mice although its expression has been reported to be downregulated in 16-month old female, compared with age-matched male, triple transgenic mice [32].

The data indicate that plaque development was coupled with microglial activation and that these changes, as well as the concentration of insoluble $A\beta_{1-42}$, were more evident in brain tissue prepared from female, compared with male, APP/PS1 mice. Importantly the evidence suggests that these changes impact on spatial learning since female APP/PS1 mice, but not their male counterparts, exhibited deficits in the reversal phase of the Morris water maze task.

Microglial activation was assessed in this study by evaluating expression of CD11b and CD68 mRNA and the data indicate that both were increased in brain tissue prepared from APP/PS1 transgenic mice compared with wildtype mice, and that expression was greater in tissue prepared from female, compared with male, APP/PS1 mice. It is known that CD11b mRNA is expressed at very low levels under resting conditions and is markedly increased when microglia are activated in response to any stress [39]. CD68 is a lysosomal marker expressed in phagocytic cells and increased expression is thought to be indicative of increased lysosomal activity [39], which accompanies phagocytosis. While CD68 expression was observed throughout the brain of APP/PS1 transgenic mice, we have observed that CD68 immunoreactivity is more marked proximal to Aβ plaques [40]. However the current literature suggests that minimal phagocytosis of plaques occurs [41] and therefore one possibility is that the CD68-positive cells are involved in phagocytosis of cell debris adjacent to Aβ plaques, or perhaps smaller Aβ aggregates. Interestingly, changes in expression of IL-1β mRNA closely mirrored those in CD68 and CD11b and this may be interpreted as indicative of an inflammatory environment. There is conflicting evidence relating to the effect of an inflammatory environment on phagocytosis of Aβ with some reports suggesting that it exerts an inhibitory effect [42] and others proposing that clearance relies on a local inflammatory response [43, 44]. Whereas the present study does not address this question directly, the evidence is indicative of parallel changes in microglial activation, IL-1β expression and Aβ accumulation in APP/PS1

mice. In contrast to the changes observed in markers of microglial activation, we found that expression of the astrocytic marker, GFAP, was greater in male, compared with female, APP/PS1 transgenic mice.

There is a great deal of evidence indicating that Aβ induces microglial activation *in vitro*, that injection of Aβ induces microglial activation *in vivo* [45] and there is evidence that microglial activation occurs in the earliest stages of AD [1]. In transgenic mice which harbor the human APP gene with the Indiana and Swedish mutations, microglial activation, as assessed by CD11b immunoreactivity, coincided with the development of Aβ plaques [46]. On the other hand, it has been suggested that plaque formation precedes microglial activation, at least when assessed by MHC II immunoreactivity, in APP/PS1 mice [47].

Several studies have reported that inflammatory changes negatively impact on behavioural performance. Here, a deficit in the reversal phase of the Morris water maze task was evident only in female APP/PS1 mice, in which inflammatory changes were more profound, although the data indicate that microglial activation was also increased in brain tissue prepared from male APP/PS1 mice. Thus, some degree of neuroinflammation can be sustained by animals before an impact on spatial learning is detectable. Although there are reports of memory impairments in transgenic mouse models of AD [48, 49], this appears to be the first report of a sex bias in behaviour which is coupled with Aβ accumulation, although overexpression of COX2 in APP/PS1 mice also uncovered a deficit in spatial memory in female, compared with male, mice [50]. A sex-related spatial memory deficit has been reported in 4 month-old female 3xTg mice which is coupled with higher circulating corticosterone levels [51] and in 3 month-old female $APP_{swe}$ mice [52] though these changes preceded Aβ deposition and in both cases older female mice performed similarly to males.

We have demonstrated that the accumulation of Aβ in 9 month-old APP/PS1 mice is more profound in female, compared with male, mice and propose that this sex-related change results from a combined increase in BACE-1 expression and a decrease in IDE expression. This highlights an early change in Aβ processing in female mice. Importantly, these changes are coupled with a sex-related increase in microglial activation and a poorer performance in a spatial learning task, suggesting a possible causal relationship between these factors.

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## References


1. Cagnin, A., et al., *In-vivo measurement of activated microglia in dementia.* Lancet, 2001. **358**(9280): p. 461-7.
2. Breitner, J.C., *The role of anti-inflammatory drugs in the prevention and treatment of Alzheimer's disease.* Annu Rev Med, 1996. **47**: p. 401-11.
3. Varvel, N.H., et al., *NSAIDs prevent, but do not reverse, neuronal cell cycle reentry in a mouse model of Alzheimer disease.* J Clin Invest, 2009. **119**(12): p. 3692-702.
4. Heneka, M.T., et al., *Molecular mechanisms and therapeutic application of NSAIDs and derived compounds in Alzheimer's disease.* Curr Alzheimer Res, 2011. **8**(2): p. 115-31.
5. Griffin, W.S., et al., *Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease.* Proc Natl Acad Sci U S A, 1989. **86**(19): p. 7611-5.
6. Wood, J.A., et al., *Cytokine indices in Alzheimer's temporal cortex: no changes in mature IL-1 beta or IL-1RA but increases in the associated acute phase proteins IL-6, alpha 2-macroglobulin and C-reactive protein.* Brain Res, 1993. **629**(2): p. 245-52.
7. Cacabelos, R., et al., *Serum tumor necrosis factor (TNF) in Alzheimer's disease and multi-infarct dementia.* Methods Find Exp Clin Pharmacol, 1994. **16**(1): p. 29-35.
8. Motta, M., et al., *Altered plasma cytokine levels in Alzheimer's disease: correlation with the disease progression.* Immunol Lett, 2007. **114**(1): p. 46-51.
9. Paresce, D.M., R.N. Ghosh, and F.R. Maxfield, *Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor.* Neuron, 1996. **17**(3): p. 553-65.
10. Ard, M.D., et al., *Scavenging of Alzheimer's amyloid beta-protein by microglia in culture.* J Neurosci Res, 1996. **43**(2): p. 190-202.
11. DeWitt, D.A., et al., *Astrocytes regulate microglial phagocytosis of senile plaque cores of Alzheimer's disease.* Exp Neurol, 1998. **149**(2): p. 329-40.
12. Fiala, M., et al., *Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients.* J Alzheimers Dis, 2005. **7**(3): p. 221-32; discussion 255-62.
13. Zaghi, J., et al., *Alzheimer disease macrophages shuttle amyloid-beta from neurons to vessels, contributing to amyloid angiopathy.* Acta Neuropathol, 2009. **117**(2): p. 111-24.
14. Wang, D.S., D.W. Dickson, and J.S. Malter, *beta-Amyloid degradation and Alzheimer's disease.* J Biomed Biotechnol, 2006. **2006**(3): p. 58406.
15. Iwata, N., et al., *Presynaptic localization of neprilysin contributes to efficient clearance of amyloid-beta peptide in mouse brain.* J Neurosci, 2004. **24**(4): p. 991-8.
16. Howell, S., J. Nalbantoglu, and P. Crine, *Neutral endopeptidase can hydrolyze beta-amyloid(1-40) but shows no effect on beta-amyloid precursor protein metabolism.* Peptides, 1995. **16**(4): p. 647-52.
17. Kanemitsu, H., T. Tomiyama, and H. Mori, *Human neprilysin is capable of degrading amyloid beta peptide not only in the monomeric form but also the pathological oligomeric form.* Neurosci Lett, 2003. **350**(2): p. 113-6.
18. Liu, Y., et al., *In vitro and in vivo degradation of Abeta peptide by peptidases coupled to erythrocytes.* Peptides, 2007. **28**(12): p. 2348-55.
19. Shirotani, K., et al., *Neprilysin degrades both amyloid beta peptides 1-40 and 1-42 most rapidly and efficiently among thiorphan- and phosphoramidon-sensitive endopeptidases.* J Biol Chem, 2001. **276**(24): p. 21895-901.
20. Iwata, N., et al., *Metabolic regulation of brain Abeta by neprilysin.* Science, 2001. **292**(5521): p. 1550-2.

21. El-Amouri, S.S., et al., *Neprilysin protects neurons against Abeta peptide toxicity.* Brain Res, 2007. **1152**: p. 191-200.
22. Hemming, M.L., et al., *Reducing amyloid plaque burden via ex vivo gene delivery of an Abeta-degrading protease: a novel therapeutic approach to Alzheimer disease.* PLoS Med, 2007. **4**(8): p. e262.
23. Leissring, M.A., et al., *Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death.* Neuron, 2003. **40**(6): p. 1087-93.
24. Marr, R.A., et al., *Neprilysin gene transfer reduces human amyloid pathology in transgenic mice.* J Neurosci, 2003. **23**(6): p. 1992-6.
25. Kurochkin, I.V., *Amyloidogenic determinant as a substrate recognition motif of insulin-degrading enzyme.* FEBS Lett, 1998. **427**(2): p. 153-6.
26. Farris, W., et al., *Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 4162-7.
27. Sondag, C.M., G. Dhawan, and C.K. Combs, *Beta amyloid oligomers and fibrils stimulate differential activation of primary microglia.* J Neuroinflammation, 2009. **6**: p. 1.
28. Brookmeyer, R., S. Gray, and C. Kawas, *Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset.* Am J Public Health, 1998. **88**(9): p. 1337-42.
29. Hy, L.X. and D.M. Keller, *Prevalence of AD among whites: a summary by levels of severity.* Neurology, 2000. **55**(2): p. 198-204.
30. Vina, J. and A. Lloret, *Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-beta peptide.* J Alzheimers Dis, 2010. **20 Suppl 2**: p. S527-33.
31. Qiu, C., M. Kivipelto, and E. von Strauss, *Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention.* Dialogues Clin Neurosci, 2009. **11**(2): p. 111-28.
32. Hirata-Fukae, C., et al., *Females exhibit more extensive amyloid, but not tau, pathology in an Alzheimer transgenic model.* Brain Res, 2008. **1216**: p. 92-103.
33. Wang, J., et al., *Gender differences in the amount and deposition of amyloidbeta in APPswe and PS1 double transgenic mice.* Neurobiol Dis, 2003. **14**(3): p. 318-27.
34. Maynard, C.J., et al., *Gender and genetic background effects on brain metal levels in APP transgenic and normal mice: implications for Alzheimer beta-amyloid pathology.* J Inorg Biochem, 2006. **100**(5-6): p. 952-62.
35. Placanica, L., L. Zhu, and Y.M. Li, *Gender- and age-dependent gamma-secretase activity in mouse brain and its implication in sporadic Alzheimer disease.* PLoS One, 2009. **4**(4): p. e5088.
36. Schafer, S., et al., *Gender dependent APP processing in a transgenic mouse model of Alzheimer's disease.* J Neural Transm, 2007. **114**(3): p. 387-94.
37. Miners, J.S., et al., *Abeta-degrading enzymes in Alzheimer's disease.* Brain Pathol, 2008. **18**(2): p. 240-52.
38. Yasojima, K., et al., *Reduced neprilysin in high plaque areas of Alzheimer brain: a possible relationship to deficient degradation of beta-amyloid peptide.* Neurosci Lett, 2001. **297**(2): p. 97-100.
39. Lynch, M.A., *The multifaceted profile of activated microglia.* Mol Neurobiol, 2009. **40**(2): p. 139-56.
40. Gallagher, J.J., et al., *Modest Amyloid Deposition is Associated with Iron Dysregulation, Microglial Activation, and Oxidative Stress.* J Alzheimers Dis, 2012. **28**(1): p. 147-61.
41. Meyer-Luehmann, M., et al., *Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease.* Nature, 2008. **451**(7179): p. 720-4.

42. Koenigsknecht-Talboo, J. and G.E. Landreth, *Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines.* J Neurosci, 2005. **25**(36): p. 8240-9.
43. Weiner, H.L. and D. Frenkel, *Immunology and immunotherapy of Alzheimer's disease.* Nat Rev Immunol, 2006. **6**(5): p. 404-16.
44. Shaftel, S.S., et al., *Sustained hippocampal IL-1beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology.* J Clin Invest, 2007. **117**(6): p. 1595-604.
45. Walsh, D.M., et al., *Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo.* Nature, 2002. **416**(6880): p. 535-9.
46. Dudal, S., et al., *Inflammation occurs early during the Abeta deposition process in TgCRND8 mice.* Neurobiol Aging, 2004. **25**(7): p. 861-71.
47. Gordon, M.N., et al., *Time course of the development of Alzheimer-like pathology in the doubly transgenic PS1+APP mouse.* Exp Neurol, 2002. **173**(2): p. 183-95.
48. Puolivali, J., et al., *Hippocampal A beta 42 levels correlate with spatial memory deficit in APP and PS1 double transgenic mice.* Neurobiol Dis, 2002. **9**(3): p. 339-47.
49. Cao, D., et al., *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, 2007. **282**(50): p. 36275-82.
50. Melnikova, T., et al., *Cycloxygenase-2 activity promotes cognitive deficits but not increased amyloid burden in a model of Alzheimer's disease in a sex-dimorphic pattern.* Neuroscience, 2006. **141**(3): p. 1149-62.
51. Clinton, L.K., et al., *Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice.* Neurobiol Dis, 2007. **28**(1): p. 76-82.
52. King, D.L., et al., *Progressive and gender-dependent cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease.* Behav Brain Res, 1999. **103**(2): p. 145-62.

**Figure Legends**

**Figure 1. Female APP/PS1 mice display deficits in spatial learning.**

No gender- or genotype-related changes were observed in the training phase of the Morris water maze task (A). In the reversal phase of the task, the mean latency in finding the platform was increased in female mice (B). Genotype was a significant factor in the performance of female mice only (latency, $^{**}p<0.001$; F(1,21.06); 2-way ANOVA) leading to female APP/PS1 (n=5) mice exhibiting impaired performance on day 4. Male APP/PS1 transgenic (n=5) and wildtype mice (n=7) and female wildtype mice (n=11) performed similarly.

**Figure 2. Evidence of enhanced glial activation in female APP/PS1 mice.**

Expression of CD11b (A), CD68 (B) and IL-1 mRNA (D) were significantly enhanced in hippocampal and cortical tissue prepared from female, compared with male, mice ($^{+}p<0.047$; F(1,4.729; $^{+}p<0.0175$; F(1,6.476) (+p<0.0041; F(1,15.82); respectively; 2-way ANOVA; n=4) and significantly greater in both male and female APP/PS1 mice compared with wildtype ($^{*}p<0.0101$; F(1,7.739); ($^{*}p<0.0001$; F(1,52.36); respectively; 2-way ANOVA; n=4). GFAP mRNA (C) expression was increased in brain tissue from APP/PS1 compared with wildtype mice ($^{*}p<0.0001$; F(1,54.09); 2-way ANOVA; n=4) however female mice revealed a decrease in GFAP mRNA expression in comparison to male mice ($^{+}p<0.0048$; F(1,13.02); 2-way ANOVA; n=4).

**Figure 3. Female APP/PS1 mice display greater Aβ plaque burden than male APP/PS1 mice.**

Congo red staining of Aβ identified a greater number of plaques in hippocampus and cortex of female compared with male mice (A). Quantification of plaque number (B) revealed an enhanced Aβ plaque burden in female compared with male APP/PS1 mice ($^{*}p<0.05$; Student's t-test for Independent means; n=4). Data are expressed as the mean plaque number on 4 coronal brain sections from each animal. Analysis of soluble (C) and insoluble (D) Aβ1-42 in brain tissue (containing hippocampus and cortex) revealed a significant increase in peptide concentrations in APP/PS1 mice compared with wildtype mice ($^{*}p<0.05$; ANOVA; n=4). The concentration of insoluble Aβ1-42 (D) was significantly enhanced in brain tissue from female compared with male APP/PS1 mice ($^{*}p<0.05$; ANOVA; n=4).

**Figure 4. Enhanced BACE-1 and decreased IDE expression in female APP/PS1 mice.**

Neprilysin mRNA expression (A) was unchanged in brain tissue containing hipocampus and cortex from male and female mice. There was a significant increase in BACE-1 mRNA expression in brain tissue prepared from female, compared with male mice (C; $^{*}p<0.0077$; F(1,13.64); 2-way ANOVA; n=4) although no genotype-related changes were identified. There was a significant decrease in IDE mRNA expression in tissue prepared from female APP/PS1 mice compared with female wildtype mice (B; $^{*}p<0.0162$; F(1,9.215); 2-way ANOVA; n=4) and a gender x genotype interaction in female APP/PS1 mice (Fig 4B; $^{*}p<0.0258$; (F1,7.454; 2-way ANOVA; n=4).