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# Sex- and brain region-specific acceleration of $\beta$ -amyloidogenesis following behavioral stress in a mouse model of Alzheimer's disease

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## Abstract

**Background:** It is hypothesized that complex interactions between multiple environmental factors and genetic factors are implicated in sporadic Alzheimer's disease (AD); however, the underlying mechanisms are poorly understood. Importantly, recent evidence reveals that expression and activity levels of the  $\beta$ -site APP cleaving enzyme 1 (BACE1), which initiates amyloid- $\beta$  ( $A\beta$ ) production, are elevated in AD brains. In this study, we investigated a molecular mechanism by which sex and stress interactions may accelerate  $\beta$ -amyloidogenesis and contribute to sporadic AD.

**Results:** We applied 5-day restraint stress (6 h/day) to the male and female 5XFAD transgenic mouse model of AD at the pre-pathological stage of disease, which showed little amyloid deposition under non-stressed control conditions. Exposure to the relatively brief behavioral stress increased levels of neurotoxic  $A\beta_{42}$  peptides, the  $\beta$ -secretase-cleaved C-terminal fragment (C99) and plaque burden in the hippocampus of female 5XFAD mice but not in that of male 5XFAD mice. In contrast, significant changes in the parameters of  $\beta$ -amyloidosis were not observed in the cerebral cortex of stressed male or female 5XFAD mice. We found that this sex- and brain region-specific acceleration of  $\beta$ -amyloidosis was accounted for by elevations in BACE1 and APP levels in response to adverse stress. Furthermore, not only BACE1 mRNA but also phosphorylation of the translation initiation factor eIF2 $\alpha$  (a proposed mediator of the post-transcriptional upregulation of BACE1) was elevated in the hippocampus of stressed female 5XFAD mice.

**Conclusions:** Our results suggest that the higher prevalence of sporadic AD in women may be attributable to the vulnerability of female brains (especially, the hippocampus) to stressful events, which alter APP processing to favor the  $\beta$ -amyloidogenesis through the transcriptional and translational upregulation of BACE1 combined with elevations in its substrate APP.

## Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in the elderly. One of the hallmark pathologies of AD is the senile plaque that is constituted of amyloid- $\beta$  ( $A\beta$ ) peptides. Although mutations in three different genes favoring the overproduction of  $A\beta$  are known to cause early-onset familial AD (FAD) [1,2], the etiology of sporadic AD that accounts for the majority of AD cases remains unclear. It is hypothesized that complex

interactions between the genetic background and various environmental factors underlie sporadic AD [3,4] and a stressful lifestyle may represent one of the important risk factors for AD [5]. Elderly individuals prone to psychological distress are more likely to develop AD than those not prone to distress, and this trait is also associated with a more rapid progression of disease [6,7]. Consistent with the clinical observations, recent studies demonstrated that exposure to adverse behavioral stress accelerates the development of amyloid pathology and worsens memory decline in transgenic mouse models of AD [8-11], although the underlying molecular mechanisms have not been investigated in detail.

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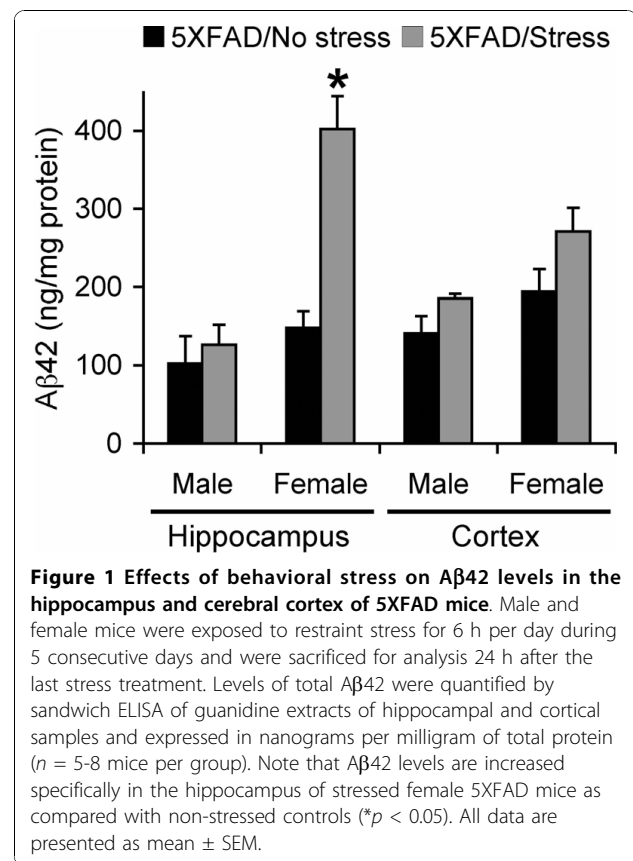
Meanwhile, it is also known that women have a higher risk of developing AD than do men. Although the longevity effect might be a factor in the preponderance of women with AD, the sex difference in AD prevalence remains even after age adjustment [12-14]. Interestingly, not only the sex but also the brain region represents a determinant of neuronal responses to stressful experience. For example, the hippocampus is a structure highly sensitive to stressor and is enriched with glucocorticoid receptors [15]. Furthermore, opposite effects of stress on hippocampal functions have been reported; an acute stressful event facilitates learning and increases dendritic spine density in male rats, whereas learning and spine density deteriorate after exposure to the same stressor in female rats [16-18]. Therefore, it is important to determine whether and how gender and adverse stress may interact to modify disease progression in different brain regions of AD transgenic mice.

The  $\beta$ -cleavage of amyloid precursor protein (APP) by BACE1 ( $\beta$ -site APP cleaving enzyme 1) initiates the generation of neurotoxic A $\beta$  peptides. Notably, evidence is accumulating that increased levels of cerebral BACE1 and/or APP expression may be crucial contributing factors in developing sporadic AD [19-22]. In this study, we tested the hypothesis that behavioral stress may trigger the upregulation of BACE1 and/or APP, which may lead to differential acceleration of  $\beta$ -amyloidogenesis in the hippocampus and cerebral cortex of male and female subjects. Specifically, 5XFAD transgenic model mice at 3 months of age, which exhibit little or only faint amyloid pathology under normal conditions [23], were exposed to 5-day restraint stress, and we compared levels of BACE1, APP, its  $\beta$ -cleavage products and plaque burden between stressed and non-stressed subjects. We clearly demonstrate that the hippocampus of female 5XFAD mice shows the dramatic acceleration of  $\beta$ -amyloidogenesis with significantly elevated levels of both BACE1 and APP expression following the relatively brief stress treatment, providing a molecular basis for the higher prevalence and incidence of sporadic AD in women. Furthermore, our results also suggest that not only transcriptional but also translational mechanisms through phosphorylation of eukaryotic initiation factor-2 $\alpha$  (eIF2 $\alpha$ ) may underlie BACE1 elevation associated with adverse stress during AD progression.

## Results

### Behavioral stress accelerates A $\beta$ accumulation in 5XFAD mice in a sex- and brain region-dependent manner

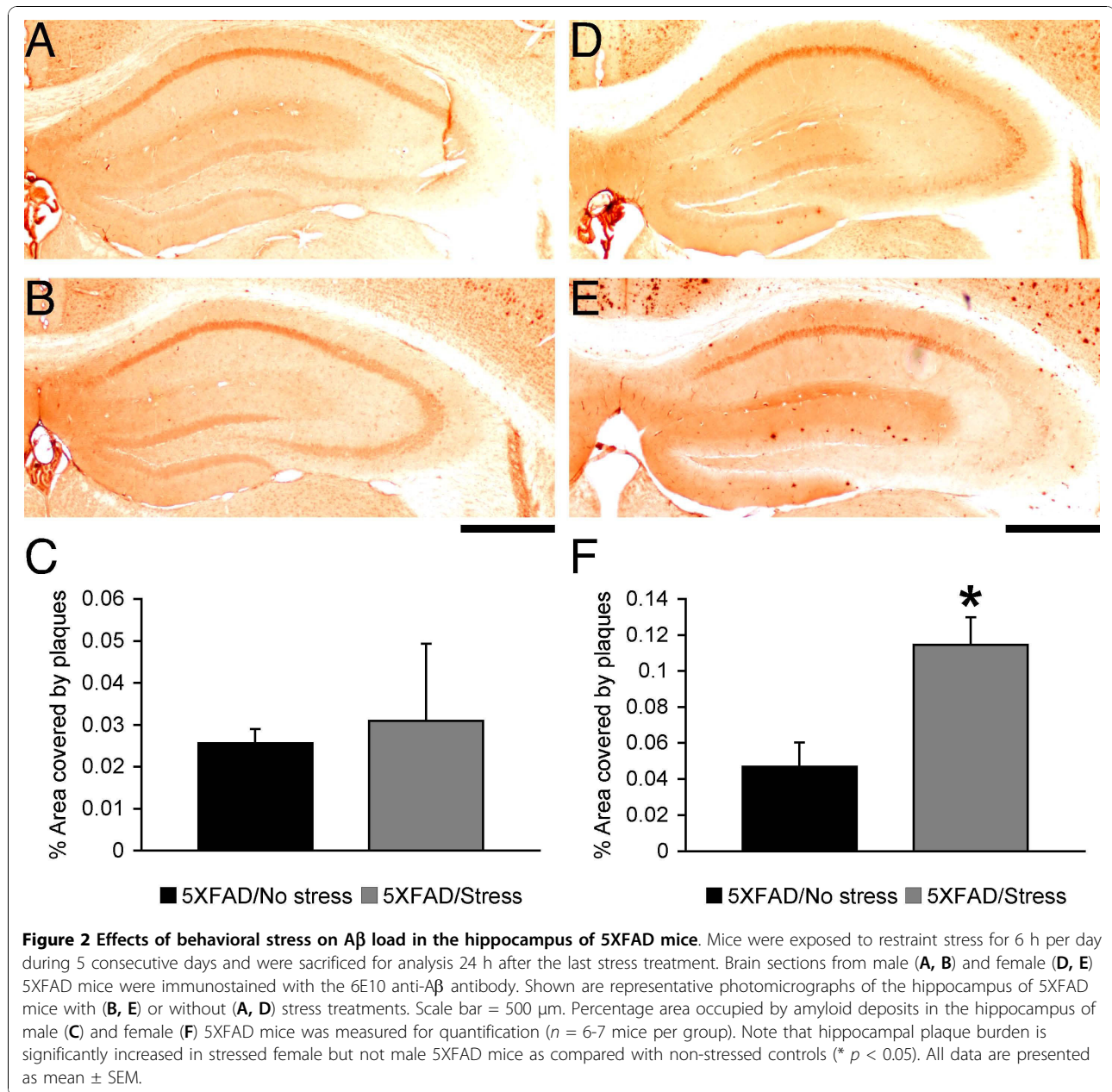
To examine the effects of adverse behavioral stress on the pathogenesis of AD, male and female 5XFAD mice at 3 months of age, which develop little or only slight amyloid deposition under normal conditions [23], were subjected to 5-day restraint stress (6 h/day). Twenty-four hours



**Figure 1** Effects of behavioral stress on A $\beta$ 42 levels in the hippocampus and cerebral cortex of 5XFAD mice. Male and female mice were exposed to restraint stress for 6 h per day during 5 consecutive days and were sacrificed for analysis 24 h after the last stress treatment. Levels of total A $\beta$ 42 were quantified by sandwich ELISA of guanidine extracts of hippocampal and cortical samples and expressed in nanograms per milligram of total protein ( $n = 5-8$  mice per group). Note that A $\beta$ 42 levels are increased specifically in the hippocampus of stressed female 5XFAD mice as compared with non-stressed controls (\* $p < 0.05$ ). All data are presented as mean  $\pm$  SEM.

after the cessation of stress treatments, we first performed sandwich ELISA to compare A $\beta$  levels between stressed and non-stressed 5XFAD mouse brains (Figure 1). The hippocampal and cortical samples were collected separately from male and female 5XFAD mice. The 5-day restraint stress significantly increased A $\beta$ 42 levels only in the hippocampus of female 5XFAD mice [ $F(1,14) = 29.00, p < 0.0001$ ], while the other three groups of 5XFAD brain samples showed no significant difference in A $\beta$ 42 levels between stressed and non-stressed subjects (Figure 1).

To test whether the ELISA measurements of A $\beta$ 42 reflected the acceleration of amyloid plaque formation, we conducted A $\beta$  immunostaining using separate groups of male and female 5XFAD mice with or without stress treatments (Figure 2). Little or no amyloid deposition was found in the hippocampus of non-stressed male (Figure 2A) or female (Figure 2D) 5XFAD control mice. Importantly, plaque load, as measured by percentage area occupied by A $\beta$  deposits, was significantly increased by exposure to the restraint stress in the hippocampus of female 5XFAD mice [ $F(1,12) = 10.96, p = 0.0062$ ] (Figure 2E, F) but not in that of male 5XFAD mice (Figure 2B, C). In the cerebral cortex, non-stressed male and female 5XFAD mice exhibited faint amyloid deposition (mainly,

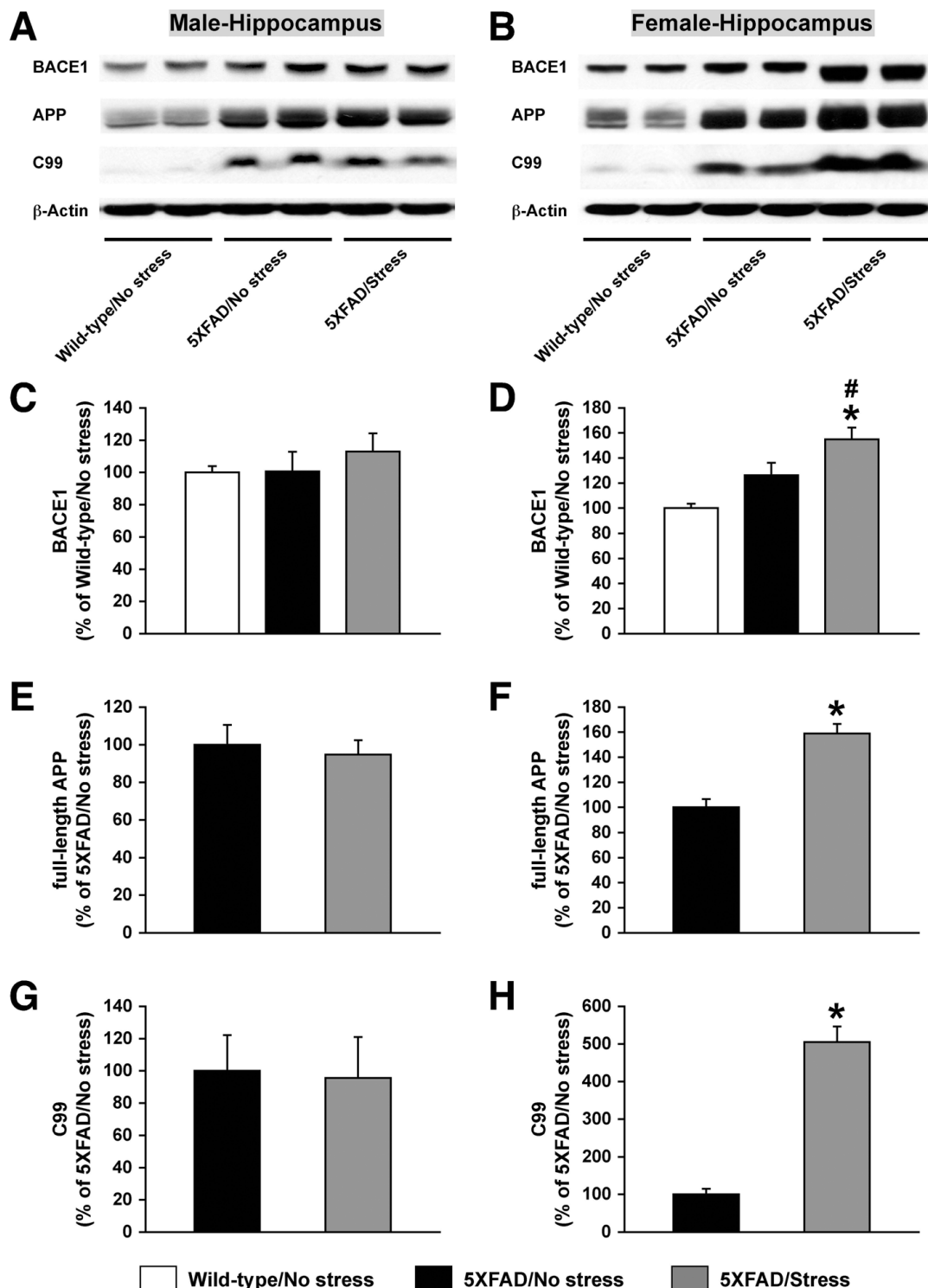


in layer 5); however, stress treatments did not affect cortical A $\beta$  burden in male or female 5XFAD mice (data not shown). Taken together, 5-day restraint stress elevated A $\beta$ 42 concentrations (~2.7-fold), leading to the higher plaque load (~2.4-fold) specifically in the hippocampus of female 5XFAD mice.

#### Behavioral stress elevates BACE1 and APP levels in 5XFAD mice in a sex- and brain region-dependent manner

To address the mechanisms underlying sex- and brain region-specific acceleration of A $\beta$  accumulation following exposure to adverse stress in 5XFAD mice, we first

compared stress-induced changes in hippocampal  $\beta$ -amyloidogenic APP processing between male and female 5XFAD mice (Figure 3). Immunoblot analysis of hippocampal homogenates from male (Figure 3A) and female (Figure 3B) mice demonstrated that 5-day exposure to restraint stress did not change BACE1 levels in male 5XFAD mice (Figure 3C), whereas it increased BACE1 expression in female 5XFAD mice (Figure 3D). A one-way ANOVA and *post-hoc* Fisher's PLSD test revealed that BACE1 levels in the hippocampus of stressed female 5XFAD mice were significantly higher than those of non-stressed wild-type and 5XFAD mice



**Figure 3** Effects of behavioral stress on APP processing in the hippocampus of 5XFAD mice. Mice were exposed to restraint stress for 6 h per day during 5 consecutive days and were sacrificed for analysis 24 h after the last stress treatment. Representative immunoblots of protein extracts from hippocampal homogenates of male (A) and female (B) 5XFAD mice with and without exposure to stress are shown along with those of non-stressed wild-type controls. Intensities of immunoreactive bands were quantified by phosphorimaging and were expressed as percentage of wild-type controls for BACE1 (C, D) or as percentage of non-stressed 5XFAD levels for full-length APP (E, F) and C99 (G, H). Data are presented as mean  $\pm$  SEM for male (C, E, G) and female (D, F, H) subjects ( $n = 4-9$  mice per group). #  $p < 0.05$  vs. non-stressed wild-type controls, \*  $p < 0.05$  vs. non-stressed 5XFAD mice.

[ $F(2,18) = 10.40, p = 0.001$ ]. Transgene-derived overexpression of human APP in 5XFAD mice was several folds relative to endogenous levels of APP detected in wild-type controls (Figure 3A, B). Similar to changes in BACE1 levels, the stress treatment significantly elevated levels of hippocampal full-length APP in female 5XFAD mice [ $F(1,14) = 32.47, p < 0.0001$ ] (Figure 3F) but not in male 5XFAD mice (Figure 3E). In consequence, levels of the  $\beta$ -secretase-cleaved C-terminal fragment (C99) were not altered in the hippocampus of stressed male 5XFAD mice (Figure 3G), while C99 levels were dramatically elevated (~5-fold) in that of stressed female 5XFAD mice compared with non-stressed 5XFAD controls [ $F(1,14) = 67.21, p < 0.0001$ ] (Figure 3H). We also tested how the same 5-day restraint stress would affect APP processing in the cerebral cortex of male and female 5XFAD mice. In contrast to changes observed in the hippocampus, the adverse behavioral stress did not significantly affect cortical BACE1 or full-length APP levels in male or female 5XFAD mice (data not shown). Collectively, 5-day exposure to restraint stress induced a marked increase of C99 levels, which was accompanied by significant elevations in both BACE1 and APP expression, specifically in the hippocampus of female 5XFAD mice consistent with the significantly increased A $\beta$ 42 concentrations and plaque burden (Figures 1 and 2).

#### **Transcriptional and translational mechanisms underlie sex- and brain region-dependent BACE1 elevation in 5XFAD mice following behavioral stress**

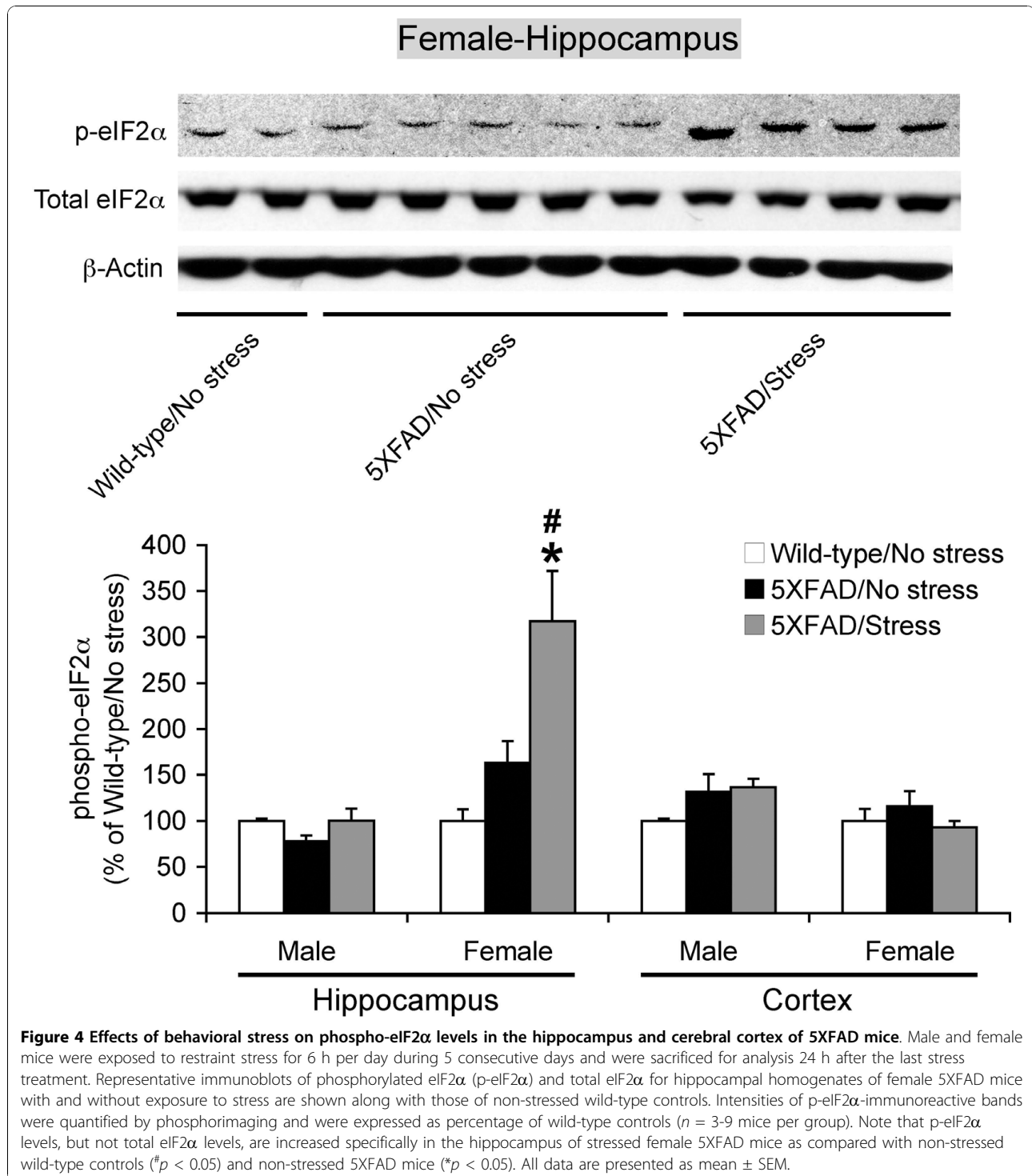
Recent evidence suggests that phosphorylation of the translation initiation factor eIF2 $\alpha$  (phospho-eIF2 $\alpha$ ) plays an important role in mediating the post-transcriptional upregulation of BACE1 in sporadic AD and 5XFAD mouse brains at advanced stages of disease that develop massive amyloid pathology [24-26]. We first examined whether the phospho-eIF2 $\alpha$  pathway was involved in the behavioral stress-induced BACE1 elevation in 5XFAD model mice (Figure 4). The baseline levels of phospho-eIF2 $\alpha$  in non-stressed 5XFAD mice at the pre-pathological stage were not significantly different from those of wild-type control mice irrespective of sex or brain regions. In parallel with changes in BACE1 expression, 5-day restraint stress significantly increased phospho-eIF2 $\alpha$  levels without affecting total eIF2 $\alpha$  levels in the hippocampus of female 5XFAD mice [ $F(2,22) = 8.70, p = 0.0016$ ]. In contrast, the other three groups of 5XFAD brain samples showed no significant changes in phospho-eIF2 $\alpha$  levels between stressed and non-stressed animals. Moreover, qPCR analysis revealed that BACE1 mRNA levels were also significantly higher in the hippocampus of female 5XFAD mice exposed to behavioral stress as compared with that of non-stressed controls

[ $F(1,6) = 20.00, p = 0.0042$ ] (Figure 5). Taken collectively, the results clearly indicate that both transcriptional and translational mechanisms through activation of the eIF2 $\alpha$  phosphorylation pathway underlie the female hippocampus-specific elevation of BACE1 expression in response to behavioral stressors in the 5XFAD model.

#### **Discussion**

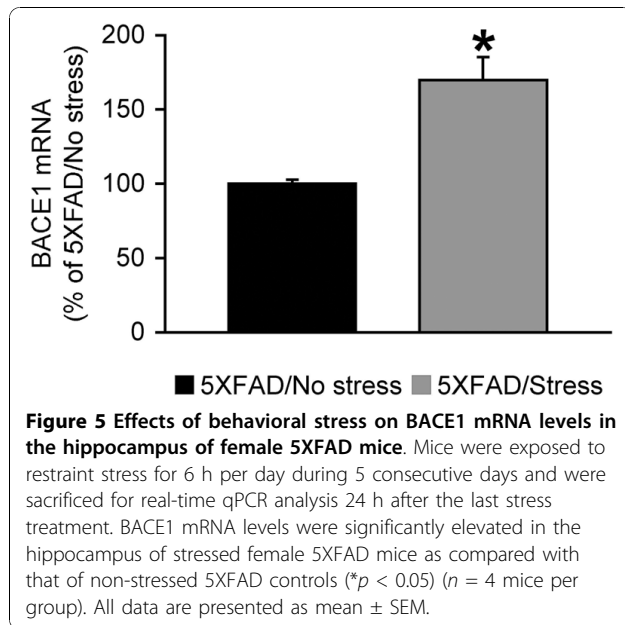
Very few AD cases can be attributable to genetic causes, while the etiology of sporadic AD that constitutes the majority of AD cases remains unclear [3,4]. Nevertheless, most transgenic models of AD are created based on a simple genetic association between the rare inherited form of FAD and excessive A $\beta$  production and do not encompass acquired characteristics [27-30]. Many non-genetic factors including age, lifestyle (e.g., daily stress and diets), medical history and education have been reported to contribute to increasing the risk for AD [31,32]. Epidemiological investigations also show gender differences in the incidence and prevalence of AD with females being at higher risk [12-14], although a biological foundation for gender differences remains to be determined. In this study, we applied a relatively brief behavioral stress to male and female 5XFAD transgenic model mice at the pre-pathological stage of disease that shows little or only faint amyloid deposition, and tested the hypothesis that sex and stress interactions may represent a key mechanism underlying sporadic AD and rendering women more prone to develop AD.

Our results clearly demonstrated that 5-day exposure to restraint stress increased levels of A $\beta$ 42 peptides, a pathogenic A $\beta$  species that is more hydrophobic and has the propensity to assemble into neurotoxic oligomers and aggregates [33-35], in the hippocampus of female 5XFAD mice but not in male 5XFAD mice. Meanwhile, the same stress treatment did not significantly affect A $\beta$ 42 levels in the cerebral cortex of male or female 5XFAD mice. Accordingly, amyloid plaque formation was also accelerated specifically in the female 5XFAD hippocampus following restraint stress. Interestingly, previous studies applied much longer stress treatments (e.g., several months of immobilization and/or isolation) to APP transgenic mice and showed that such prolonged stress exposures resulted in elevated A $\beta$  concentrations and pathology in both the hippocampus and cortex without distinction based on sex [8-11]. Therefore, it should be noted that only 5-day exposure to restraint stress was sufficient to significantly elevate levels of A $\beta$ 42 and plaque load in 5XFAD mice in this study. Of particular importance, such a brief stress treatment revealed that the hippocampus of females is vulnerable and prone to develop amyloid deposits in response to adverse behavioral stressors. These findings support the idea that the



higher prevalence of sporadic AD in women may be, at least in part, attributable to the vulnerability of female brain, especially the hippocampus, to stress mechanisms that favor  $\beta$ -amyloidogenic processing of APP. This is also consistent with the observation that hippocampal A $\beta$  deposition is one of the earliest features of AD [36].

What mechanisms underlie the sex- and brain region-specific acceleration of A $\beta$  accumulation following brief stress exposure? Previous studies demonstrate a sex difference in stress effects and estrogens are known to potentiate the glucocorticoid secretion under stress conditions [37,38]. This mechanism may represent the key



component of the stress response that promotes  $\beta$ -amyloidosis more profoundly in female 5XFAD brains. Alternatively, recent studies have started to reveal the involvement of gender-specific molecular signaling processes or sex chromosome gene expression in addition to estrogen effects in causing sex differences in neuronal function [39,40]. Further study is needed to address the precise mechanisms including interactions between sex hormones and different stress mediators linking with changes in neuronal amyloidogenic processing of APP associated with AD.

Importantly, we found that BACE1 expression was elevated specifically in the hippocampus of stressed female 5XFAD mice in accordance with increased levels of A $\beta$ 42 and plaque burden. It has been reported that protein and/or activity levels of BACE1 become elevated in brains of sporadic AD patients [19-21,41] and 5XFAD mice [26,42-44] as disease progresses into the severer pathological stage with established amyloid plaques. Recent studies including ours demonstrate that phosphorylation of the translation initiation factor eIF2 $\alpha$  plays a critical role in mediating the post-transcriptional upregulation of BACE1 associated with AD [25,26]. Increases in phospho-eIF2 $\alpha$  levels occur in brains of sporadic AD and advanced pathological stages of APP transgenic mice including the 5XFAD model [24-26,45,46] and are shown to correlate with BACE1 elevation [25,26]. In the present study, baseline phospho-eIF2 $\alpha$  levels of non-stressed 5XFAD mice at the pre-pathological stage were not significantly different from those of wild-type control mice, while they were increased by 5-day restraint stress exposure specifically

in the hippocampus of female 5XFAD mice in line with BACE1 elevation. Therefore, these results suggest that the phospho-eIF2 $\alpha$ -dependent translational upregulation of BACE1 in response to behavioral stressors may represent an important molecular mechanism by which environmental factors initiate  $\beta$ -amyloidogenesis before significant A $\beta$  deposition occurs during the early phase of sporadic AD. This hypothesis is strongly supported by our recent observation that the increase in phospho-eIF2 $\alpha$  induced by Sal 003, a specific inhibitor of its phosphatase, elevates BACE1 levels in younger 5XFAD mice, which have not yet showed BACE1 upregulation at basal levels concomitant with only marginal increases in eIF2 $\alpha$  phosphorylation [26].

In addition to the translational mechanism, transcriptional control of BACE1 may also be implicated in AD pathogenesis [47,48]. In this study, we showed the increase of BACE1 mRNA level in the hippocampus of stressed female 5XFAD mice compared with that of non-stressed controls, suggesting a possibility that transcriptional mechanisms may also contribute to the BACE1 elevation associated with adverse behavioral stress. Our results are consistent with the findings that the promoter region of BACE1 gene contains glucocorticoid responsive elements [49] and that glucocorticoid administration facilitates A $\beta$  production possibly via increases in transcription of the BACE1 gene through this binding site [50]. Some studies with postmortem human brains report elevations in BACE1 mRNA levels associated with sporadic AD [21,51], while others show no changes in mRNA despite the increased levels of BACE1 activity and protein [22,52-54]. Therefore, the mechanisms underlying BACE1 elevation in the sporadic AD brain remain controversial, which may be accounted for by differences in complex environmental factors mainly responsible for the disease progression. Our mouse model study suggests that both transcriptional and translational mechanisms may underlie BACE1 elevations associated with adverse stress during the development of AD.

Intriguingly, we also found that stress-responsive increases in APP expression levels occurred only in the hippocampus of female 5XFAD mice. Therefore, it seems likely that elevations in both BACE1 and its substrate APP work cooperatively to enable dramatic increases in the intermittent  $\beta$ -cleaved C-terminal fragment C99 and the acceleration of A $\beta$ 42 production and plaque formation in the hippocampus of female 5XFAD mice following 5-day exposure to behavioral stress. Given that A $\beta$  and C99 peptides are amyloidogenic and can induce synaptic failure, neurodegeneration and memory loss [34,55-59], behavioral stress-dependent elevations in both  $\beta$ -cleavage products through BACE1

and APP upregulation have important implications for the pathogenesis of sporadic AD and the progression of neuronal dysfunction. Our findings are in agreement with recent reports showing that exposure to stress-level glucocorticoids (daily injections of dexamethasone for 7-21 days) elevates both BACE1 and APP levels leading to accelerated C99 production and A $\beta$  accumulation [50,60,61]. However, these pharmacologically induced stress responses seem more robust than the behavioral stress regimen applied in this study as the changes are observed in brains of male subjects including 3xTg-AD transgenic model mice and middle-aged wild-type mice or rats. In any case, our present study combined with others indicates that elevated levels of glucocorticoids found in sporadic AD brains [62-64] may not only be a consequence of the pathology but also play a causal role in triggering  $\beta$ -amyloidogenesis through BACE1 and APP elevations during earlier stages of disease progression.

### Conclusions

Our mouse model study clearly demonstrates that the responsiveness of brains (especially, hippocampal neurons) to stress conditions, which shift APP processing toward  $\beta$ -amyloidogenesis by upregulating BACE1 and its substrate APP, represents a crucial contributing factor in the development of sporadic AD and may account for a mechanism underlying the increased prevalence of women to develop AD. Moreover, our data also suggest that transcriptional and translational mechanisms may underlie BACE1 elevation in response to adverse stressors, supporting the idea that therapeutic interventions aimed at suppressing stress-related signaling pathways (e.g., reduction of glucocorticoids or eIF2 $\alpha$  phosphorylation) may be beneficial for slowing down AD progression.

### Methods

#### Animals

We used 5XFAD transgenic mice that co-overexpress FAD mutant forms of human APP (the Swedish mutation: K670N, M671L; the Florida mutation: I716V; the London mutation: V717I) and presenilin-1 (PS1: M146L, L286V) transgenes under transcriptional control of the neuron-specific mouse Thy-1 promoter (Tg6799 line) [23,65]. 5XFAD lines (B6/SJL genetic background) were maintained by crossing hemizygous transgenic mice with B6/SJL F1 breeders (Taconic, Hudson, NY). 5XFAD transgenic mice used were hemizygotes with respect to the transgene and non-transgenic wild-type littermate mice served as controls. Genotyping was performed by PCR analysis of tail DNA. All experiments were done blind with respect to the genotype of the mice, and were conducted with the approval of the Nathan Kline Institute Animal Care and Use Committee.

### Behavioral stress

Stressed 5XFAD mice were individually placed in a well-ventilated plastic tube (Diameter: 3.8 cm) (541-RR, Plas-Labs, Lansing, MI) and restrained for 6 h per day during 5 consecutive days. After each stress session, the mice were returned to their cage where they were housed in isolation with free access to food and water. Twenty-four hours after the last exposure to restraint stress, brain samples were collected. The timing of stress treatments was arranged so that 5XFAD mice were precisely at 3 months of age when sacrificed. Non-stressed control mice were kept group-housed in their home cage and were sacrificed for analysis at 3 months of age.

### A $\beta$ 42 ELISA

Sandwich A $\beta$  ELISA was performed as described previously [66,67]. Briefly, each hemibrain sample was extracted in 8X cold 5 M guanidine HCl plus 50 mM Tris HCl (pH 8.0) buffer, and centrifuged at 20,000 g for 1 h at 4°C to remove insoluble material. Final guanidine HCl concentrations were below 0.1 M. Protein concentrations were determined by a BCA protein assay kit (Pierce, Rockford, IL). To quantitate total levels of cerebral A $\beta$ 42, supernatant fractions were analyzed by a well-established human A $\beta$ 42 ELISA kits (KHB3441, Invitrogen, Carlsbad, CA) according to the protocol of the manufacturer. Optical densities at 450 nm of each well were read on a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA), and sample A $\beta$ 42 concentrations were determined by comparison with the standard curves. A $\beta$ 42 concentration values were normalized to total brain protein concentrations and expressed in nanograms per milligram of total protein.

### A $\beta$ immunohistochemistry

Mice were transcardially perfused with 4% paraformaldehyde in phosphate buffered saline (PBS) under deep isoflurane anesthesia. The brain was removed and sectioned coronally at 40  $\mu$ m on a vibratome (VT1200, Leica Microsystems, Wetzlar, Germany), and successive sections were stored in PBS containing 0.01% sodium azide at 4°C. Four sections per mouse were stained by the avidin-biotin peroxidase complex method for immunohistochemical analysis of amyloid deposition in the hippocampus and cerebral cortex [66,67]. Each section was separated by ~120  $\mu$ m and taken at levels of -1.5/-1.9 mm to bregma according to the mouse brain atlas of Franklin and Paxinos [68]. The sections were incubated overnight at 4°C with monoclonal anti-A $\beta$ 1-16 antibody (1:200; 6E10, Signet, Dedham, MA). The ABC kit (PK-2200, Vector Laboratories, Burlingame, CA) was utilized with 3,3'-diaminobenzidine tetrahydrochloride as a chromogen to visualize the reaction product. The sections were then mounted on charged slides, dehydrated in a



series of alcohol, cleared in xylene, and covered with a coverslip. Light microscopy was conducted on an Axioskop 2 microscope equipped with an AxioCaM HRc digital camera (Zeiss, Munich, Germany) for capturing images. Semi-quantitative analysis was performed using AxioVision imaging software with the AutoMeasure module (Zeiss). Identified objects after thresholding were individually inspected to confirm the object as a plaque or not in a blinded manner. Percentage area occupied by A $\beta$  deposits in the hippocampus and cortex was assessed bilaterally, and the average of the individual measurements from each mouse was calculated to compare plaque load between the stressed and non-stressed 5XFAD mice.

#### Immunoblot analysis

Hippocampal and cortical samples were taken from the mice under deep isoflurane anesthesia and were snap-frozen for Western blot analysis [66,67]. Each sample was homogenized in 5 volumes of modified RIPA buffer containing 150 mM NaCl, 50 mM Tris HCl (pH 8.0), 1 mM EDTA, 1% IGEPAL, 0.5% sodium deoxycholate, 0.1% SDS and protease/phosphatase inhibitor cocktails (Calbiochem, La Jolla, CA), and centrifuged at 10,000 g for 10 min to remove any insoluble material. Protein concentrations were determined by a BCA kit (Pierce), and 20-50  $\mu$ g of protein was run on 4-12% NuPAGE gels (Invitrogen) and transferred to nitrocellulose membrane. After blocking, membranes were probed with anti-BACE1 (1:1,000, MAB5308, Millipore, Billerica, MA), an antibody that recognizes C-terminal epitope in APP (1:1,000, C1/6.1, kindly provided by Dr. Paul Mathews, Nathan Kline Institute) to detect full-length APP/C-terminal fragments, anti-phospho-eIF2 $\alpha$ (Ser51) (1:1,000, #3398, Cell Signaling Technology, Danvers, MA), anti-eIF2 $\alpha$  (1:1,000, #9722, Cell Signaling Technology) or anti- $\beta$ -actin (1:15,000, AC-15, Sigma, St. Louis, MO). They were then incubated with horseradish peroxidase-conjugated secondary IgG. Immunoblot signals were visualized by an ECL chemiluminescence substrate reagent kit (Pierce) and were quantified by densitometric scanning and image analysis using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

#### Real-time qPCR

qPCR was performed in triplicate on frozen hippocampal samples as described previously [69,70]. TaqMan qPCR primers were utilized for mouse BACE1 mRNA (Mm00478671\_m1, Applied Biosystems, Foster City, CA) and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Mm99999915\_g1, Applied Biosystems). Samples were assayed on a real-time qPCR cyler (7900HT, Applied Biosystems) in 96-well optical plates covered with optical adhesive film.

Standard curves and cycle threshold were generated using standards obtained from total mouse brain RNA. The delta delta cycle threshold (ddCT) method was employed to determine relative gene level differences between stressed and non-stressed 5XFAD mice with GAPDH qPCR products used as a control, and expression levels were presented as percentage of non-stress controls. Negative controls consisted of the reaction mixture without input RNA.

#### Statistical analysis

The significance of differences between the groups was determined by a one-way ANOVA and *post-hoc* Fisher's PLSD tests were performed when appropriate. Data were presented as mean  $\pm$  SEM and the level of significance was set for *p* value less than 0.05.

#### Acknowledgements

This work was supported by National Institutes of Health grant R01 MH067251 (M.O.) and Alzheimer's Association grant IIRG-08-91231 (M.O.).

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#### Authors' contributions

LD performed a majority of the experiments, analyzed the data and wrote the manuscript. MJA and SDG performed the qPCR experiment and analyzed the data. MO designed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

Received: 28 July 2010 Accepted: 8 November 2010

Published: 8 November 2010

#### References

1. Hardy J: Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci* 1997, **20**:154-159.
2. Selkoe DJ: Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001, **81**:741-766.
3. Blennow K, de Leon MJ, Zetterberg H: Alzheimer's disease. *Lancet* 2006, **368**:387-403.
4. Wu J, Basha MR, Zawia NH: The environment, epigenetics and amyloidogenesis. *J Mol Neurosci* 2008, **34**:1-7.
5. Rothman SM, Mattson MP: Adverse stress, hippocampal networks, and Alzheimer's disease. *Neuromolecular Med* 2010, **12**:56-70.
6. Wilson RS, Barnes LL, Bennett DA, Li Y, Bienias JL, Mendes de Leon CF, Evans DA: Proneness to psychological distress and risk of Alzheimer disease in a biracial community. *Neurology* 2005, **64**:380-382.
7. Wilson RS, Arnold SE, Schneider JA, Kelly JF, Tang Y, Bennett DA: Chronic psychological distress and risk of Alzheimer's disease in old age. *Neuroepidemiology* 2006, **27**:143-153.
8. Jeong YH, Park CH, Yoo J, Shin KY, Ahn SM, Kim HS, Lee SH, Emson PC, Suh YH: Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV7171-CT100 transgenic mice, an Alzheimer's disease model. *FASEB J* 2006, **20**:729-731.
9. Dong H, Goico B, Martin M, Csernansky CA, Bertchume A, Csernansky JG: Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience* 2004, **127**:601-609.

10. Dong H, Yuede CM, Yoo HS, Martin MV, Deal C, Mace AG, Csernansky JG: Corticosterone and related receptor expression are associated with increased  $\beta$ -amyloid plaques in isolated Tg2576 mice. *Neuroscience* 2008, **155**:154-163.
11. Lee KW, Kim JB, Seo JS, Kim TK, Im JY, Baek IS, Kim KS, Lee JK, Han PL: Behavioral stress accelerates plaque pathogenesis in the brain of Tg2576 mice via generation of metabolic oxidative stress. *J Neurochem* 2009, **108**:165-175.
12. Baum LW: Sex, hormones, and Alzheimer's disease. *J Gerontol A Biol Sci Med Sci* 2005, **60**:736-743.
13. Andersen K, Launer LJ, Dewey ME, Letenneur L, Ott A, Copeland JR, Dartigues JF, Kragh-Sorensen P, Baldereschi M, Brayne C, Lobo A, Martinez-Lage JM, Sijinen T, Hofman A: Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. EURODEM Incidence Research Group. *Neurology* 1999, **53**:1992-1997.
14. Hy LX, Keller DM: Prevalence of AD among whites: a summary by levels of severity. *Neurology* 2000, **55**:198-204.
15. Kim JJ, Diamond DM: The stressed hippocampus, synaptic plasticity and lost memories. *Nat Rev Neurosci* 2002, **3**:453-462.
16. Shors TJ, Chua C, Falduto J: Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. *J Neurosci* 2001, **21**:6292-6297.
17. Bangasser DA, Shors TJ: The hippocampus is necessary for enhancements and impairments of learning following stress. *Nat Neurosci* 2007, **10**:1401-1403.
18. Waddell J, Bangasser DA, Shors TJ: The basolateral nucleus of the amygdala is necessary to induce the opposing effects of stressful experience on learning in males and females. *J Neurosci* 2008, **28**:5290-5294.
19. Fukumoto H, Cheung BS, Hyman BT, Irizarry MC:  $\beta$ -Secretase protein and activity are increased in the neocortex in Alzheimer disease. *Arch Neurol* 2002, **59**:1381-1389.
20. Yang LB, Lindholm K, Yan R, Citron M, Xia W, Yang XL, Beach T, Sue L, Wong P, Price D, Li R, Shen Y: Elevated  $\beta$ -secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nat Med* 2003, **9**:3-4.
21. Li R, Lindholm K, Yang LB, Yue X, Citron M, Yan R, Beach T, Sue L, Sabbagh M, Cai H, Wong P, Price D, Shen Y: Amyloid  $\beta$  peptide load is correlated with increased  $\beta$ -secretase activity in sporadic Alzheimer's disease patients. *Proc Natl Acad Sci USA* 2004, **101**:3632-3637.
22. Matsui T, Ingelsson M, Fukumoto H, Ramasamy K, Kowa H, Frosch MP, Irizarry MC, Hyman BT: Expression of APP pathway mRNAs and proteins in Alzheimer's disease. *Brain Res* 2007, **1161**:116-123.
23. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R: Intraneuronal  $\beta$ -amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* 2006, **26**:10129-10140.
24. Chang RC, Wong AK, Ng HK, Hugon J: Phosphorylation of eukaryotic initiation factor-2 $\alpha$  (eIF2 $\alpha$ ) is associated with neuronal degeneration in Alzheimer's disease. *Neuroreport* 2002, **13**:2429-2432.
25. O'Connor T, Sadleir KR, Maus E, Velliquette RA, Zhao J, Cole SL, Eimer WA, Hitt B, Bembinsten LA, Lammich S, Lichtenthaler SF, Hebert SS, De Strooper B, Haass C, Bennett DA, Vassar R: Phosphorylation of the translation initiation factor eIF2 $\alpha$  increases BACE1 levels and promotes amyloidogenesis. *Neuron* 2008, **60**:988-1009.
26. Devi L, Ohno M: Phospho-eIF2 $\alpha$  level is important for determining abilities of BACE1 reduction to rescue cholinergic neurodegeneration and memory defects in 5XFAD mice. *PLoS One* 2010, **5**:e12974.
27. McGowan E, Eriksen J, Hutton M: A decade of modeling Alzheimer's disease in transgenic mice. *Trends Genet* 2006, **22**:281-289.
28. Gotz J, Streffer JR, David D, Schild A, Hoernl F, Pannanen L, Kurosinski P, Chen F: Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. *Mol Psychiatry* 2004, **9**:664-683.
29. Gotz J, Ittner LM: Animal models of Alzheimer's disease and frontotemporal dementia. *Nat Rev Neurosci* 2008, **9**:532-544.
30. Morrisette DA, Parachikova A, Green KN, LaFerla FM: Relevance of transgenic mouse models to human Alzheimer disease. *J Biol Chem* 2009, **284**:6033-6037.
31. McDowell I: Alzheimer's disease: insights from epidemiology. *Aging (Milano)* 2001, **13**:143-162.
32. Qiu C, Kivipelto M, von Strauss E: Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues Clin Neurosci* 2009, **11**:111-128.
33. Findeis MA: The role of amyloid  $\beta$  peptide 42 in Alzheimer's disease. *Pharmacol Ther* 2007, **116**:266-286.
34. McGowan E, Pickford F, Kim J, Onstead L, Eriksen J, Yu C, Skipper L, Murphy MP, Beard J, Das P, Jansen K, Delucia M, Lin WL, Dolios G, Wang R, Eckman CB, Dickson DW, Hutton M, Hardy J, Golde T: A $\beta$ 42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron* 2005, **47**:191-199.
35. Haass C, Selkoe DJ: Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid  $\beta$ -peptide. *Nat Rev Mol Cell Biol* 2007, **8**:101-112.
36. de Leon MJ, DeSanti S, Zinkowski R, Mehta PD, Pratico D, Segal S, Clark C, Kerkman D, DeBernardis J, Li J, Lair L, Reisberg B, Tsui W, Rusinek H: MRI and CSF studies in the early diagnosis of Alzheimer's disease. *J Intern Med* 2004, **256**:205-223.
37. Sotiropoulos I, Cerqueira JJ, Catania C, Takashima A, Sousa N, Almeida OF: Stress and glucocorticoid footprints in the brain-the path from depression to Alzheimer's disease. *Neurosci Biobehav Rev* 2008, **32**:1161-1173.
38. Weiser MJ, Handa RJ: Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic-pituitary-adrenal axis via estrogen receptor alpha within the hypothalamus. *Neuroscience* 2009, **159**:883-895.
39. Mizuno K, Giese KP: Towards a molecular understanding of sex differences in memory formation. *Trends Neurosci* 2010, **33**:285-291.
40. Andreano JM, Cahill L: Sex influences on the neurobiology of learning and memory. *Learn Mem* 2009, **16**:248-266.
41. Ahmed RR, Holler CJ, Webb RL, Li F, Beckett TL, Murphy MP: BACE1 and BACE2 enzymatic activities in Alzheimer's disease. *J Neurochem* 2010, **112**:1045-1053.
42. Ohno M, Cole SL, Yasvoina M, Zhao J, Citron M, Berry R, Disterhoft JF, Vassar R: BACE1 gene deletion prevents neuron loss and memory deficits in 5XFAD APP/PS1 transgenic mice. *Neurobiol Dis* 2007, **26**:134-145.
43. Zhao J, Fu Y, Yasvoina M, Shao P, Hitt B, O'Connor T, Logan S, Maus E, Citron M, Berry R, Binder L, Vassar R:  $\beta$ -Site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: implications for Alzheimer's disease pathogenesis. *J Neurosci* 2007, **27**:3639-3649.
44. Zhang X-M, Cai Y, Xiong K, Cai H, Luo X-G, Feng J-C, Clough RW, Struble RG, Patrylo PR, Yan X-X:  $\beta$ -Secretase-1 elevation in transgenic mouse models of Alzheimer's disease is associated with synaptic/axonal pathology and amyloidogenesis: implications for neuritic plaque development. *Eur J Neurosci* 2009, **30**:2271-2283.
45. Page G, Rioux Bilan A, Ingrand S, Lafay-Chebassier C, Pain S, Perault Pochat MC, Bouras C, Bayer T, Hugon J: Activated double-stranded RNA-dependent protein kinase and neuronal death in models of Alzheimer's disease. *Neuroscience* 2006, **139**:1343-1354.
46. Kim HS, Choi Y, Shin KY, Joo Y, Lee YK, Jung SY, Suh YH, Kim JH: Swedish amyloid precursor protein mutation increases phosphorylation of eIF2 $\alpha$  in vitro and in vivo. *J Neurosci* 2007, **27**:1528-1537.
47. Rossner S, Sastre M, Bourne K, Lichtenthaler SF: Transcriptional and translational regulation of BACE1 expression - implications for Alzheimer's disease. *Prog Neurobiol* 2006, **79**:95-111.
48. Wen Y, Yu WH, Maloney B, Bailey J, Ma J, Marie I, Maurin T, Wang L, Figueroa H, Herman M, Krishnamurthy P, Liu L, Planel E, Lau LF, Lahiri DK, Duff K: Transcriptional regulation of  $\beta$ -secretase by p25/cdk5 leads to enhanced amyloidogenic processing. *Neuron* 2008, **57**:680-690.
49. Sambamurti K, Kinsey R, Maloney B, Ge YW, Lahiri DK: Gene structure and organization of the human  $\beta$ -secretase (BACE1) promoter. *FASEB J* 2004, **18**:1034-1036.
50. Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM: Glucocorticoids increase amyloid- $\beta$  and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci* 2006, **26**:9047-9056.

51. Coulson DT, Beyer N, Quinn JG, Brockbank S, Hellemans J, Brent G, Ravid R, Johnston JA: **BACE1 mRNA Expression in Alzheimer's Disease Postmortem Brain Tissue.** *J Alzheimers Dis* 2010.
52. Holsinger RM, McLean CA, Beyreuther K, Masters CL, Evin G: **Increased expression of the amyloid precursor  $\beta$ -secretase in Alzheimer's disease.** *Ann Neurol* 2002, **51**:783-786.
53. Borghi R, Patriarca S, Traverso N, Piccini A, Storace D, Garuti A, Gabriella C, Patrizio O, Massimo T: **The increased activity of BACE1 correlates with oxidative stress in Alzheimer's disease.** *Neurobiol Aging* 2007, **28**:1009-1014.
54. Preece P, Virley DJ, Costandi M, Coombes R, Moss SJ, Mudge AW, Jazin E, Cairns NJ:  **$\beta$ -Secretase (BACE) and GSK-3 mRNA levels in Alzheimer's disease.** *Brain Res Mol Brain Res* 2003, **116**:155-158.
55. Geula C, Wu CK, Saroff D, Lorenzo A, Yuan M, Yankner BA: **Aging renders the brain vulnerable to amyloid  $\beta$ -protein neurotoxicity.** *Nat Med* 1998, **4**:827-831.
56. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ: **Amyloid- $\beta$  protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory.** *Nat Med* 2008, **14**:837-842.
57. Nalbantoglu J, Tirado-Santiago G, Lahsaini A, Poirier J, Goncalves O, Verge G, Momoli F, Welner SA, Massicotte G, Julien JP, Shapiro ML: **Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein.** *Nature* 1997, **387**:500-505.
58. Choi SH, Park CH, Koo JW, Seo JH, Kim HS, Jeong SJ, Lee JH, Kim SS, Suh YH: **Memory impairment and cholinergic dysfunction by centrally administered A $\beta$  and carboxyl-terminal fragment of Alzheimer's APP in mice.** *FASEB J* 2001, **15**:1816-1818.
59. Lee KW, Im JY, Song JS, Lee SH, Lee HJ, Ha HY, Koh JY, Gwag BJ, Yang SD, Paik SG, Han PL: **Progressive neuronal loss and behavioral impairments of transgenic C57BL/6 inbred mice expressing the carboxy terminus of amyloid precursor protein.** *Neurobiol Dis* 2006, **22**:10-24.
60. Catania C, Sotiropoulos I, Silva R, Onofri C, Breen KC, Sousa N, Almeida OF: **The amyloidogenic potential and behavioral correlates of stress.** *Mol Psychiatry* 2009, **14**:95-105.
61. Li WZ, Li WP, Yao YY, Zhang W, Yin YY, Wu GC, Gong HL: **Glucocorticoids increase impairments in learning and memory due to elevated amyloid precursor protein expression and neuronal apoptosis in 12-month old mice.** *Eur J Pharmacol* 2010, **628**:108-115.
62. Swanwick GR, Kirby M, Bruce I, Buggy F, Coen RF, Coakley D, Lawlor BA: **Hypothalamic-pituitary-adrenal axis dysfunction in Alzheimer's disease: lack of association between longitudinal and cross-sectional findings.** *Am J Psychiatry* 1998, **155**:286-289.
63. Masugi F, Ogihara T, Sakaguchi K, Otsuka A, Tsuchiya Y, Morimoto S, Kumahara Y, Saeki S, Nishide M: **High plasma levels of cortisol in patients with senile dementia of the Alzheimer's type.** *Methods Find Exp Clin Pharmacol* 1989, **11**:707-710.
64. Csernansky JG, Dong H, Fagan AM, Wang L, Xiong C, Holtzman DM, Morris JC: **Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia.** *Am J Psychiatry* 2006, **163**:2164-2169.
65. Ohno M, Chang L, Tseng W, Oakley H, Citron M, Klein WL, Vassar R, Disterhoft JF: **Temporal memory deficits in Alzheimer's mouse models: rescue by genetic deletion of BACE1.** *Eur J Neurosci* 2006, **23**:251-260.
66. Kimura R, Devi L, Ohno M: **Partial reduction of BACE1 improves synaptic plasticity, recent and remote memories in Alzheimer's disease transgenic mice.** *J Neurochem* 2010, **113**:248-261.
67. Devi L, Ohno M: **Genetic reductions of  $\beta$ -site amyloid precursor protein-cleaving enzyme 1 and amyloid- $\beta$  ameliorate impairment of conditioned taste aversion memory in 5XFAD Alzheimer's disease model mice.** *Eur J Neurosci* 2010, **31**:110-118.
68. Franklin KBJ, Paxinos G: *The Mouse Brain in Stereotaxic Coordinates* New York: Academic Press; 2008.
69. Alldred MJ, Che S, Ginsberg SD: **Terminal Continuation (TC) RNA amplification enables expression profiling using minute RNA input obtained from mouse brain.** *Int J Mol Sci* 2008, **9**:2091-2104.
70. Ginsberg SD: **Transcriptional profiling of small samples in the central nervous system.** *Methods Mol Biol* 2008, **439**:147-158.

doi:10.1186/1756-6606-3-34

**Cite this article as:** Devi et al.: Sex- and brain region-specific acceleration of  $\beta$ -amyloidogenesis following behavioral stress in a mouse model of Alzheimer's disease. *Molecular Brain* 2010 **3**:34.

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