Modulation of Alzheimer's Disease Pathology and Neuroplasticity

by Environmental Factors

BY

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THESIS

Submitted as partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Anatomy and Cell Biology
in the Graduate College of the
University of Illinois at Chicago, 2012

Chicago, Illinois

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This thesis is dedicated in the loving memory of my Grandma, whom I love and respect very much. I also would like to dedicate this thesis to my parents and my siblings, without their endless loves and supports I would not have achieved everything I have today. Last but not least, to my beloved husband, *Patrick*, whose enduring love and support have given me the strength to keep moving forward.

ACKNOWLEDGEMENTS

This work would not be accomplished without the helps from so many individuals. First and foremost, I thank my advisor, Dr. Orly Lazarov, for her supports all these years and for making me a better scientist. I thank my committee chairperson, Dr. Ernesto Bongarzone, for being such a great listener. I value every pieces of advices and encouragements that he has given me. I also thank my thesis committee- Drs. Scott Brady, Elliott Mufson and John Kessler for their continuous inputs and supports, guiding me through my thesis work. It's truly my honor to have all of you serving on my thesis committee, and I have learned so much from each of you. I thank my mentor in Taiwan, Dr. Fan-Lu Kung, for being the source of inspiration to pursue my PhD study in the AD field. I thank Drs. James Unnerstall and Conwell Anderson for showing me their passions and dedications to medical teaching. Special thank to Drs. John Larson and Peng Xu, for being such a great collaborator and for their works on electrophysiology for us. I also would like to thank the department of Anatomy and Cell Biology for giving me this precious opportunity to pursue my PhD study here in the US, and for providing such a resourceful and friendly scientific environment for conducting my research studies.

To members of the Lazarov lab: Archana Gadadhar, Bich Duong, Yafit Kuttner and Mike Demars: We have been through so much together, I really appreciate all the scientific inputs, emotional supports, laughs and cries and friendship that you have shared with me. You guys are the reasons that I can still smile at the end of the day.

I thank the members of the department of Anatomy and Cell Biology, who had helped me along the way: Drs. Gustavo Pigino and Gerardo Morfini for all the discussions, their scientific advices and generous lab resources. I am grateful to Maria Carlstrom, Mike Driscoll, Linda Johnston and Rory Sullivan for their helps with all the administrative matters.

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ACKNOWLEDGEMENTS (continued)

I am also very lucky to make so many great friends at UIC: Kasia Pituch, Sun Kyong Lee, Hongling Zhu, Viviana Ulloa Jofré, Amy Hebert, Agnieszka Kaminska, Aurora Lopez-Rosas, Ana Lis Moyano, Evelyn Nwabuisi, Chang You and Bin Wang. Thank you for all the memorable moments we shared together. It would never be the same without all of you. And special thanks to Hongling, Ludovico, and Yuka for their helps with the experiments and trouble shootings along the way. To all the friends that I have made in Chi-town, I thank you for helping me paint such a colorful chapter of life in Chicago.

Finally, I would like to thank my family. I could never express enough gratitude toward my parents for believing in me and having so much faith and confidence in what I do. I thank them for never giving up on me and for offering me the freedom and autonomy to pursue my own dreams. I thank my siblings, Joyce, Jessica and Jeffrey, for making me a better big sister. I would not be able to accomplish any of these without all of you. And I thank my in-laws for their full supports of the every decisions that we have made.

Last but not least, I am deeply grateful for my husband, Patrick, for helping me always find my way home. I thank him for standing by me through the toughest time of my life and for making me stronger and better every day. You are the best.

I love you all.

YH

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LIST OF ABBREVIATIONS

18S rRNA	18 Small Ribosomal RNA
Aβ	Beta Amyloid
AD	Alzheimer's Disease
Akt	Also known as Protein Kinase B (PKB)
AMPA	α-Amino-3-Hydroxy-5-Methylisoxazole-4-Propionic Acid
APP	Amyloid Precursor Protein
APPswe/PS1ΔE9	APP Swedish mutation and PS1 Deletion at Exon 9
A.U.	Arbitrary Units
BDNF	Brain-derived Neurotrophic Factor
bFGF	Basic Fibroblast Growth Factor
BrdU	5'-Bromo-2'-Deoxyuridine
BSA	Bovine Serum Albumin
CaMKII	Calcium/Calmodulin-Dependent Protein Kinase II
CaMKIV	Calcium/Calmodulin-Dependent protein kinase IV
CBP	CREB-Binding Protein
cDNA	Complementary DNA
CK2	Casein Kinase 2
CREB	cAMP Response Element-Binding Protein
DCX	Doublecortin
DG	Dentate Gyrus
EE	Environmental Enrichment
EGF	Epidermal Growth Factor
ELISA	Enzyme-Linked Immunosorbent Assay

FAD	Familial Alzheimer's Disease
fEPSPs	Field Excitatory Postsynaptic Potentials
GADPH	Glyceraldehyde 3-Phosphate Dehydrogenase
GFAP	Glial Fibrillary Acid Protein
GH	Group-Housed
GluR1	Glutamate Receptor 1
GSK3β	Glycogen Synthase Kinase 3β
hr	Hour
IGF-1	Insulin Growth Factor-1
KHC	Kinesin-1 Heavy Chain
KLC	Kinesin-1 Light Chain
LTP	Long Term Potentiation
mAb	Monoclonal Antibody
mRNA	Messenger RNA
NeuN	Neuronal Nuclei
NFTs	Neurofibrillary Tangles
NGF	Nerve Growth Factor
NMDAR	N-Methyl-D-Aspartate Receptor
NonTg	Nontransgenic
NPC	Neural Progenitor Cell
NSC	Neural Stem Cell
NT-3	Neurotrophin-3
pAb	Polyclonal Antibody

LIST OF ABBREVIATIONS (continued)

PCNA   Proliferating Cell Nuclear Antigen

PKC   Protein Kinase C

PS1   Presenilin-1

PSD-95   Postsynaptic Density Protein 95

RT-PCR   Reverse Transcriptase-Polymerase Chain Reaction

S100β   S100 Calcium Binding Protein B

SE   Standard Error of Means

SGL   Subgranular Layer

SH   Standard Housing

Sox2   SRY (Sex Determining Region Y)-Box 2

SVZ   Subventricular Zone

TBS   Tris-Buffered Saline

tPA   Tissue-Type Plasminogen Activator

SUMMARY

This study provides significant evidence to demonstrate that environmental elements and lifestyle are crucial factors contributing to both the development and prevention of Alzheimer's disease (AD). We have shown that experience in an enriched environment rescues impaired neurogenesis, ameliorates disease pathology and promotes neuronal survival in a mouse model of familial Alzheimer's disease (FAD).

AD is an age-related, progressive brain disease that is characterized by memory loss and cognitive decline, which severely interferes with daily activities and social life. There is no cure for AD and methods for prevention are still being sought. A definitive diagnosis of AD can only be made post-mortem in brain autopsies upon the detection of two pathological hallmarks, i.e. amyloid plaques and neurofibrillary tangles.

While the search for treatment is a major goal of research studies, exploring ways of preventing or retarding disease progression is equally desirable. Rare, familial forms (FAD) are caused by mutations in amyloid precursor protein (APP), presenilin 1 (PS1), or presenilin 2 (PS2). However, the majority of AD cases are the sporadic late-onset form of the disease, for which the greatest risk factor is aging. This strongly suggests that environmental factors may play a role in the development and/or progression of the disease. In support of this notion, epidemiological studies have reported decreased risks of AD in populations with higher levels of education and in those who regularly exercise and are frequently engaged in social activities. Strong support for this notion comes from studies in AD animal models that show behavioral improvement in cognitive tests following experience in an enriched environment.

To elucidate the role of environmental factors in modulating AD pathology and brain plasticity, we examined the effect of environmental enrichment (EE) on brain structure and

SUMMARY (continued)

function in the well-characterized FAD-linked APPswe/PS1ΔE9 mouse model. For this purpose, young mice were exposed to an enriched environment immediately after weaning. They were group-housed in large cages filled with running wheels, colorful tunnels and toys. We then examined neuroplasticity and compared pathological hallmarks of the disease in the brains of "enriched" mice with the brains of mice that were maintained in standard laboratory conditions.

We have shown that the experience of mice in an enriched environment promotes neurogenesis, as manifested by an increased number of new neurons incorporated in the granule layer of the dentate gyrus, and by the restored ability of the AD brain to produce new neurons to a similar extent as healthy mice. Additionally, EE enhances hippocampal long-term potentiation (LTP) and upregulates gene expression of molecular targets associated with synaptic plasticity. This suggests an overall increase of brain plasticity and synaptic function following experience in an enriched environment.

In addition to increased brain plasticity, we observed that the experience of AD mice in an enriched environment reduces pathological hallmarks. Specifically, we are the first to show that the level of soluble oligomeric Aβ, the neurotoxic precursor of amyloid plaques, is significantly reduced in the brains of "enriched" mice, as is amyloid deposition, which we have shown in our previous studies. Furthermore, this is also the first study to show that EE can effectively decrease the level of hyperphosphorylated tau, the precursor of neurofibrillary tangles in the brains of these mice. Finally, we observed an upregulation of the anterograde motor protein kinesin-1 in the brains of enriched mice, suggesting that axonal transport is enhanced following EE.

SUMMARY (continued)

The molecular pathways mediating these beneficial effects of EE on brain plasticity and pathology are not fully understood. Thus, we aimed to elucidate the molecular mechanism(s) underlying the effects of EE on AD pathology. We have shown that the levels of brain-derived neurotrophic factor (BDNF) and BDNF-associated molecular targets are significantly elevated in the hippocampi of mice following EE, suggesting an increase of EE-induced BNDF-dependent signal transduction. Of particular significance is the upregulation of cAMP Response Element-Binding Protein (CREB), which plays a major role in the formation of long-term memory. Additionally, we have identified defective signaling pathways in FAD mice that could not be rescued by EE. EE has shown to modulate the expression levels of GSK3β and Akt kinases in nontransgenic mice, but not in FAD-linked APPswe/PS1ΔE9 mice, suggesting regulation of a different signaling pathway by EE in FAD mice.

Taken together, this work strongly suggests that, in spite of severe neuropathology, experience in an enriched environment enhances neurogenesis, attenuates pathology and enhances synaptic plasticity in our FAD-linked APPswe/PS1ΔE9 mouse model. The primary contributions of this work have been (1) the establishment of the effects of EE on brain plasticity and pathology in AD, as well as (2) the unraveling of the molecular mechanisms underlying the effects of EE on AD brains. This study strongly supports the notion that environmental factors are critical contributors to the development and prevention of AD. This study may pioneer the development of (1) a therapeutic approach that may prevent or attenuate cognitive deficits in AD, (2) drug therapy that may target molecular signals activated by EE, and (3) neural stem cell therapy for neurodegenerative diseases.

I. INTRODUCTION

A. Overview

Alzheimer's disease (AD) was first described more than a hundred year ago by the German psychiatrist, Alois Alzheimer. Today, it is the most common type of dementia that accounts for an estimated 60-80% of all dementia cases, and it is the fifth-leading cause of death for the population aged 65 and older in the United States (Miniño et al., 2010). The elderly population is continuously increasing at a rapid rate, especially in the developed countries, such as the United States and Japan. The numbers of age-related and/or neurodegeneration-related cognitive decline cases are escalating, and the long-term consequences of this demographic change are affecting social, economical and medical status with enormous socio-economical costs. As heartbreaking as it seems, at the present time there is still no cure for AD. The structural and physiological changes in the brains of AD patients have been shown to begin many years before the appearance of clinical symptoms such as memory loss. This important observation should be taken into consideration when it comes to developing the treatments for AD. Therefore, exploring ways of preventing or retarding the disease progression is as equally important as identifying a cure for the disease.

The majority of the AD cases are sporadic, late onset form. Although the genetic cause for the sporadic form of AD is still unknown, it has been shown that aging is one of the greatest risk factor for sporadic AD. This suggests that environmental factors may contribute to the development and progression of age-related cognitive decline and AD. Maintaining an active lifestyle has been shown to delay age-dependent cognitive decline and reduces the risk of developing AD and other neurodegenerative diseases in the healthy individuals [for review, see (Lautenschlager et al., 2012)]. Epidemiological studies

also correlated the levels of education, social engagement and leisure activity with the delayed onset of mild cognitive impairments and the decreased likelihood of developing AD (Bennett et al., 2005; Evans et al., 1997; Stern et al., 1994; Wang et al., 2011). Thus, in the recent decades, increasing attention has been paid to the role of healthy lifestyle as a protective factor against age-related dementia. The beneficial effects of physical exercise, healthy diet and frequent engagement in mental and social activities have been reported on various health outcomes, most often related to the prevention of cardiovascular diseases and diabetes. However, accumulating evidence suggests that physical exercise also positively impacts cognitive function and dementia. The protective effects of mental and social activities on cognitive decline have also been reviewed elsewhere (Wang et al., 2011). Nevertheless, it has not yet been determined whether modulation of lifestyle can prevent, delay or reverse AD and to what approach will the effect be the most effective. And either has the molecular mechanisms underlying environmental effects on the development of AD been fully elucidated.

In that regard, the past decades have made tremendous efforts in trying to address questions related to the phenomenon of adult neurogenesis in the mammalian brain. Adult neurogenesis is thought to play a role in learning and memory (Gould et al., 1999a; Gould et al., 1999b). Intriguingly, physical exercise and environmental enrichment are two important factors that have been shown to effectively promote hippocampal neurogenesis in several animal models (Brown et al., 2003; Kempermann et al., 1998b; van Praag et al., 1999b). However, it has also been long debated whether brain plasticity, specifically adult neurogenesis, can be induced under neuropathological conditions, such as AD, to an extent that would reduce AD pathology, rescue brain structure and restore function. This led to a

series of experiments aimed at looking at the effects that environmental enrichment has upon brain plasticity and AD pathology.

Current research on AD relies heavily on the use of AD transgenic mouse models. Numerous AD transgenic mouse models were developed, most of which express familial Alzheimer's disease-linked mutant forms of human APP and PS1 genes. These mice are used to study the etiology, pathogenesis, disease progression, as well as to evaluate therapeutic agents to treat the disease [For review, see (Balducci and Forloni, 2011)]. In this study, we chose to use the double transgenic mouse line harboring both the APPswe and PS1ΔE9 mutant transgenes driven by a mouse PrP promotor (see method section for more details). While currently there is no mouse model that faithfully replicates AD, the APPswe/PS1ΔE9 mice are thought to exhibit behavioral symptoms and pathologic hallmarks better than other models. For example, single PS1 mutant mice show very mild cognitive impairment, exhibit no extracellular amyloid deposition and show no neurodegeneration. The co-expression of human APP and PS1 mutants accelerate plaque deposition to about 4-6 months, which normally would take at least 6-8 months to develop in other single APP mutant mice. Theses mice also show severe cognitive deficits in spatial and learning memory at older ages (Savonenko et al., 2005). The advantage of using this particular AD mouse model allows us to study amyloid pathology and follow the disease progression, beginning at a relatively young age. It also allows us to study adult neurogenesis in parallel with the disease progression.

B. Research questions

In this study, we aimed at examining the effects of environmental enrichment on the development of AD hallmarks, i.e. amyloidosis and tau hyperphosphorylation and brain plasticity, i.e. hippocampal neurogenesis, as well as synaptic physiology. In addition, we

aimed at elucidating the molecular mechanism(s) underlying the effects of EE on AD. The ultimate goal of this study is to address whether experience in an enriched environment can be used as a potential therapeutic intervention for Alzheimer's disease.

Hypothesis:

We propose an overall hypothesis that *experience in environmental enrichment promotes brain plasticity and mitigates AD pathology in the brains of transgenic mice harboring familial Alzheimer's disease-linked APPswe/PS1∆E9.*

C. Specific Aims

To answer our research questions, we designed our study according to the following Specific Aims:

1. To determine the extent of neurogenesis and AD pathology in young APPswe/PS1∆E9 mice, before the onset of amyloid deposition.

Information concerning the fate of neurogenesis in FAD mice prior to extensive brain pathology is largely scarce. Thus, determining the fate of neurogenesis early in the disease would address the critical question of whether the alterations in neurogenesis contribute to the disease development, or reflect brain response to the massive pathology later in the disease. Experiments in this Aim will examine the hypothesis *that neurogenesis is impaired early in the brains of APPswe/PS1∆E9 mice.* Experiments are designed to address the extent of proliferation, differentiation and maturation of neural progenitor cells (NPCs) in both the subgranular layer (SGL) and the subventricular zone (SVZ) of 2 months old transgenic mice harboring FAD-linked APPswe/PS1∆E9 and in neurosphere cultures derived from these mice. In addition, the extent of the precursors of amyloidosis and tau hyperphosphorylation in the neurogenic regions will be examined. These experiments will

provide critical insight as for the development of brain pathology in AD, and the role of neurogenesis in AD.

2. **To determine whether the experience in an enriched environment can modulate neurogenesis, AD pathology and synaptic plasticity in FAD-linked APPswe/PS1∆E9 mice.**

We have previously shown that experience in an enriched environment downregulates the extent of amyloid deposits. However, it is not clear whether this effect is specific for amyloidosis, or whether EE can modulate other aspects of AD pathology and plasticity. Experiments in this Aim will examine the hypothesis that *experience of FAD-linked APPswe/PS1∆E9 mice in EE would ameliorate tau hyperphosphorylation and oligomeric amyloid while inducing hippocampal neurogenesis and synaptic plasticity*. Using biochemical and immunohistochemical analyses, the experiments are designed to determine the extent of amyloidosis, tau phosphorylation and neurogenesis in the hippocampus of APPswe/PS1∆E9 mice following EE. Using electrophysiological recordings from hippocampal slices, experiments will determine the extent of long-term potentiation (LTP) in APPswe/PS1∆E9 mice following EE. This Aim will determine whether environmental factors play a role in the prevention and/or development of AD.

3. **To elucidate the molecular mechanism(s) by which environmental enrichment modulates neuroplasticity and AD pathology in FAD-linked APPswe/PS1∆E9 mice.**

3.1- To examine alterations in expression level of kinases implicated in AD pathology following EE.

Our observation that EE downregulated tau hyperphosphorylation suggests the hypothesis that tau-phosphorylation-linked kinase activity is attenuated following EE. GSK3β and CK2 both have been shown to phosphorylate tau and kinesin-1, contributing to

AD pathogenesis. Experiments in this aim will examine the expression level of GSK3β and CK2 in the brains of APPswe/PS1ΔE9 and nontransgenic mice following experience in EE. The expression levels of the kinase in the cortex and hippocampus will be determined using Western blot analysis.

3.2- To examine the modulation of BDNF signaling pathways in APPswe/PS1ΔE9 mice following EE.

Our previous microarray analysis showed that BDNF mRNA level is significantly upregulated in the brains of APPswe/PS1ΔE9 mice following EE. However, whether BDNF is altered at the protein level is unknown. Taken together with recent studies suggesting that BDNF stimulation induces rapid tau dephosphorylation in neurons, this aim will examine the hypothesis that EE upregulates BDNF levels and the molecular signalings in the APPswe/PS1ΔE9 mice and their nontransgenic littermates. BDNF levels will be analyzed by ELISA. In addition, the effect of EE on transcriptional modification of BDNF-linked signaling in the brains of APPswe/PS1ΔE9 mice will be determined by quantitative real-time RT-PCR. Achievement of these experiments will aid the determination of the molecular signaling pathways modulated by EE in our AD mice.

D. Significance of the study

Alzheimer's disease was described for the first time over one hundred year ago, and yet, cure for this devastating disease is still unavailable. The elderly population continues to increase at a rapid rate, especially in the developed countries. As a result, the number of individuals affected with late onset AD is also escalating. This demographic change has social, economical and medical long-term implications. Current preclinical and clinical researches have made a lot of efforts in evaluating different interventions, mainly pharmacological, that may help to delay age-related cognitive decline. However the efficacy

of these interventions are still questionable. In addition, because AD is an aging-linked sporadic disease, it becomes apparently clear that environmental factors may play a major role in the formation of the disease.

This study focuses on investigating the effects of environmental factors on several different aspects of AD pathology and on overall brain plasticity. Using EE experimental paradigm, we have shown that environmental stimulation may be a potentially effective intervention for promoting adult neurogenesis and ameliorating amyloid and tau pathology in APPswe/PS1ΔE9 mouse model. This is the first study to address the effect of EE on the extent of soluble oligomeric Aβ and tau hyperphosphorylation in AD mice. In addition, this study is the first to examine tau hyperphosphorylation in neural stem/progenitor cells both *in vitro* and *in vivo*. This study also provides novel information concerning the molecular mechanisms by which EE regulates AD. Specifically, we provide new information concerning the effect of EE on AD-related phosphokinase expressions in these mice. We further elucidate the roles of EE-induced upregulation of BDNF signaling in synaptic plasticity and its potential involvement in pathways regulating neuronal survival, kinase expression level and tau phosphorylation. Importantly, this study is the first to show that EE upregulated CREB in the hippocampus of these mice. This provides the first molecular link between EE and molecular pathways of cognition. The outcomes of this study will contribute to the development of therapeutics strategies for the prevention or attenuation of AD.

II.   BACKGROUND AND RELEVANT LITERATURE

A. Aging and neurodegenerative diseases

Aging is a set of changes in biological process that takes place over time. While aging is viewed as a normal process, it is generally accepted that this process is accompanied by functional regression, for example, cognitive decline. While the mechanism underlying aging is largely unknown, it is speculated that a gradual decrease in cells' efficiency to perform their physiological functions might take place over time. The examples for these cellular functions include the change in overall homeostasis, mitochondrial energy metabolism, DNA repair ability and cellular quality control. [for review, see (Yankner et al., 2008)]. Normal aging can be distinguished from pathological aging, such as those in the neurodegenerative diseases, by both the degree of impairment and the rate of cognitive decline. Normal human aging is characterized by gradual cognitive decline, which may include deficits in short-term recall memory, naming and spatial memory. Conversely, in pathological aging, such as in AD, the rate of cognitive decline is rather rapid and it may involve more global and profound deficits in memory, language, reasoning and behaviors. Structurally, the pathological aging, such as in AD, exhibits significant brain atrophy in the entorhinal cortex and in the medial temporal lobe, indicating a massive extent of neuronal loss, while in the normal aging, the extent of neuronal loss is very minimal in most regions of the brain (Burke and Barnes, 2006). An important unresolved question remained is how the normal aging brain transitions into a pathological aging brain, and leading to the development of a neurodegenerative disease. This will require a lot more and better understanding of the normal aging process before we can fully elucidate the underlying causes of pathological aging and cognitive decline.

B. Alzheimer's disease (AD)

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized by massive neuronal loss and severe cognitive decline. Clinical symptoms of AD includes impaired judgment, disorientation, confusion, behavior changes and difficulty in performing daily tasks. The pathological hallmarks of the disease are associated with extracellular amyloid-beta (Aβ) peptides aggregates and neurofibrillary tangles (NFTs), composed of the intracellular aggregates of hyperphosphorylated tau protein (McKhann et al., 2011). Aβ peptides are the catalytic products of the amyloidogenic pathway of amyloid precursor protein (APP), a type I membrane protein that undergoes sequential cleavage by β-secretase (BACE1) and γ-secretase [For review see (Selkoe, 2001)]. Presenilin-1 (PS1) and presenilin-2 (PS2) form the catalytic core of the γ-secretase complex and is responsible for this second cleavage. In the non-amyloidogenic pathway, α-secretase cleaves APP within the Aβ domain and generates soluble APP fragments (sAPPα) and prevents the formation of Aβ and carboxyl-terminal fragments. APP is further cleaved by γ-secretase. Under normal circumstance, γ-secretase cleavage of APP yields predominantly A$\beta_{1\text{-}40}$. In AD, it results in an increase of A$\beta_{1\text{-}42,43}$ and/or the increased ratio of A$\beta_{1\text{-}42,43}$/A$\beta_{1\text{-}40}$ production, which subsequently leads to Aβ peptides aggregation. However, the underlying cause of these pathological hallmarks and/or the physiological roles of APP and Aβ are still poorly understood.

AD is categorized into two different forms regardless of the identical etiology. Rare, familial early-onset forms of the disease that accounts for less than 5% of all AD cases are caused by mutations in *APP*, *PS1* and *PS2*, while the majority of the AD cases are sporadic, aging-linked late-onset forms. No genetic cause has been found for the late onset form of AD, making aging currently the number one risk factor for sporadic AD. The second risk

factor for late-onset AD is the inheritance of the $\varepsilon 4$ allele of the apolipoprotein E (ApoE) protein (Corder et al., 1993; Lahiri et al., 2004). These early findings strongly suggest a critical role for environmental factors in the development of late onset AD, and in concert with yet-to-be-determined genetic factors, it all together determine the development and age-at-onset of the disease.

Alzheimer's disease affects mainly the cortical and hippocampal regions of the brain. The visible shrinkage of cortical gyri and the hippocampi in AD patients by magnetic resonance imaging (MRI) and in the post-mortem AD brains suggests a massive loss of neurons in these brain areas. The hippocampus is one of the earliest areas to be affected, along with several specific cortical regions, such as the entorhinal and pre-frontal cortex. These brain regions play critical roles in learning and memory, behaviors and higher brain functions, such as critical thinking. More importantly, the hippocampus, specifically the subgranular layer (SGL) of the dentate gyrus, is one of the two areas in the adult brain in which neurogenesis occurs postnatally. As discussed below, adult neurogenesis plays an important role in hippocampal plasticity and function, and in the network maintenance for the hippocampal formation and the limbic system. Current literatures support the idea that enhancement of hippocampal neurogenesis can improve many learning and memory tasks (Aimone et al., 2006; Kempermann et al., 2004), whereas a decline in hippocampal neurogenesis, as observed in AD or aging, deteriorates cognitive functions. Therefore finding ways to enhance hippocampal neurogenesis in AD patients may be a potential diagnostics and therapeutic strategies for AD.

C. Adult neurogenesis

It is now generally accepted that adult neurogenesis takes place in two very specific regions of the brain: in the subventricular zone (SVZ) of the lateral ventricle and in the

subgranular layer (SGL) of the dentate gyrus (Gage et al., 2008). However, the initial seminal report of cell genesis by Joseph Altman in the mid 1960s had created quite a controversy in the field (Altman and Das, 1965). It was not until the early 1980s, when Nottebohm and colleagues published a series of study to show that neurogenesis in adult song birds is related to the appearance of seasonal songs, suggesting the functional integration of new neurons in adult brain for the first time (Alvarez-Buylla and Nottebohm, 1988; Goldman and Nottebohm, 1983; Nottebohm, 1989). Later on, the introduction of the use of nucleotide analog, 5-bromo-2'-deoxyuridine (BrdU) and retroviral as tools to study adult neurogenesis opened up a whole new world in the research field of neuroplasticity (Kuhn et al., 1996; van Praag et al., 2002). Up until now, neurogenesis has been identified across different species, including rodents, birds, monkeys and humans (Eriksson et al., 1998; Kempermann et al., 1998a; Nottebohm, 1989; Pencea et al., 2001a). Continuous production of new neurons in the adult brains opened up the new potentials for therapeutic interventions for neurodegenerative diseases.

It has been reported that in young rats, there are approximately 9,000 new born cells in the SGL and up to 30,000 new born cells in the SVZ produced daily, and about half of these cells survive and integrate into the functional circuitries of the CNS (Cameron and McKay, 2001; Lois and Alvarez-Buylla, 1994). Three different types of cells in SVZ have been identified: Type B-mitotically quiescent neural stem cells that express glial fibrillary acidic protein (GFAP, subtype of astrocytes), Type C-transit amplifying, fast-proliferating cells and type A-migrating neuroblasts (see **Figure 1**) (Doetsch et al., 1999; Lazarov et al., 2010). These cells can be identified by their expression of lineage-specific markers at different stages. Newly generated cells extend throughout the length of the lateral ventricle and are migrated into the rostral migratory stream (RMS) leading to the olfactory bulb, where they differentiate into two types of inhibitory interneurons: the GABAergic granule

and periglomerular cells that functionally integrate into existing circuitry (Belluzzi et al., 2003; Carleton et al., 2003).

It has been shown that Sox2-positive nonradial cells can also give rise to new neurons and glia in the SGL (Suh et al., 2007). These radial and nonradial glial cells generate neural progenitor cells, which later give rise to neuroblasts (Palmer et al., 2000; Seri et al., 2001). The three different cell types in the SGL are: type I NSCs, type II and type III progenitor cells (see **Figure 1**). GFAP-positive radial glial-like cells (Type III neuroblast) are thought to be the cell type that gives rise to new neurons. In SGL, newly generated progenitor cells are differentiated into excitatory granule neurons and migrate locally into the granule cell layer. They further extend the dendrites to the molecular layer and the axon along the mossy fiber path, and functionally integrate in the circuitries of the dentate gyrus (Ge et al., 2006; Jessberger and Kempermann, 2003; van Praag et al., 2002).

D. Factors regulating neurogenesis

Neurogenesis involves several very crucial steps: cell division, cell differentiation, cell maturation and cell migration into functional circuits (Kempermann et al., 2003; Kronenberg et al., 2003). Natural-occurring cell apoptosis is also a common element of the neurogenic regions. It's been reported that in both SGL and SVZ, there is more than 50% reduction in the number of BrdU-labeled cells detected over a period of three months, suggesting that there is a very dynamic and very tightly-regulated cell genesis and cell death in the SVZ and SGL (Dayer et al., 2003; Winner et al., 2000). Multiple signaling pathways and multiple molecular factors are responsible for the regulation and maintenance of these newly born neurons.

Factors regulating neurogenesis include extrinsic factors such as Notch, Wnt and bone morphogenetic proteins (BMPs). BMP and Notch signaling components are both
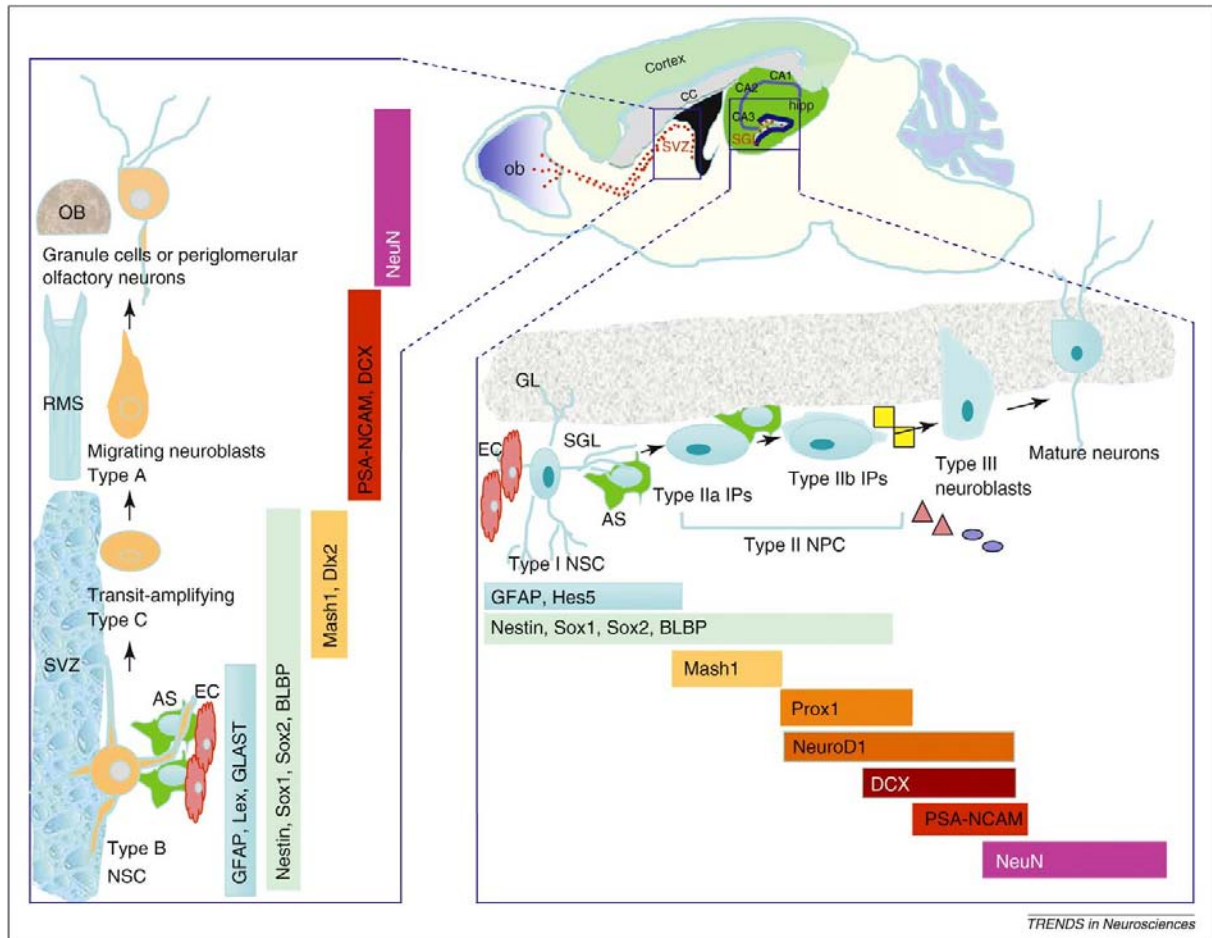
FIGURE 1 Neurogenesis in the adult rodent brain.

Sagittal section of mouse brain showing the neurogenic microenvironments in the adult brain: the subventricular zone (SVZ) and the subgranular layer (SGL) of the dentate gyrus (DG). Stages of morphological and physiological development of neural stem cells (NSCs) in the SVZ (left) and SGL (right) are illustrated in inserts. Specifically, the SGL contains type I NSCs, and type II and type III progenitor cells which can be identified by distinct morphological and molecular markers. Type I have radial processes extending into the inner molecular layer. This pool of NSCs stays relatively stable throughout life. Type II neural progenitor cells (NPCs) or intermediate progenitors (IP) have only short processes if any, and do not express GFAP. Type II cells could arise from type I cells. Type II cells can be divided to two subpopulations: (i) type IIa, and (ii) type IIb cells. They continue to proliferate and can give rise to type III cells, which are migratory neuroblasts. After a limited number of cell divisions, type III cells exit the cell cycle and become mature granule neurons. In the SVZ, type B cells resemble SGL type I cells. They give rise to GFAP negative transit-amplifying type C cells, which then give rise to type A cells that migrate radially on "glial tubes" in the rostral migratory stream (RMS) to layers in the olfactory bulb (OB) before terminal differentiation. Abbreviations: cc, corpus callosum; hipp, hippocampus. [Adapted from (Lazarov et al., 2010)].

present in the SVZ neurogenic niche, and they have been shown to be important for maintenance of astrocytic stem cell state as well as promoting SVZ self-renewal (Givogri et al., 2006; Lim and Alvarez-Buylla, 1999; Nyfeler et al., 2005). In relation to SGL neurogenesis, it has been shown that BMP signaling mediates the effects of exercise on hippocampal neurogenesis and cognition in mice, and blockage of BMP signaling by Noggin has been shown to promote hippocampal cell proliferation and self-renewal (Bonaguidi et al., 2008; Gobeske et al., 2009). Currently, little is known about Wnt Signaling in the SVZ other than the fact that Wnt 3a and Wnt5 promote SVZ neuronal proliferation and differentiation *in vitro*. However, Wnt 3 is expressed in the SGZ hippocampal astrocytes, and it is a principal regulator of hippocampal neurogenesis both *in vitro* and *in vivo* (Lie et al., 2005).

The balance of trophic support is also essential for regulating neurogenesis and cell turnover. Various growth factors, neurotrophic factors, neurotransmitters, cytokines and hormones all have been shown to affect neurogenesis one way or the other [for detailed review, see (Gage et al., 2008)] . The neurotrophins in general are positive regulators of adult neurogenesis (Frielingsdorf et al., 2007; Shimazu et al., 2006). BDNF receives most attention for its central role in promoting cell differentiation and survival in the SVZ and hippocampus (Rossi et al., 2006; Zigova et al., 1998). Previous study also showed positive correlation between the plasma BDNF level and cognitive function in aging women, implicating BDNF as a potential biomarker for diagnosing memory impairment and assessing general cognitive function (Komulainen et al., 2008).

Interestingly, the effect of fibroblast growth factor-2 (FGF-2) on neurogenesis is concentration-dependent. At low concentration (2ng/mL), FGF-2 promotes neuronal differentiation and survival *in vitro* (Walicke et al., 1986). However, at higher concentration (10 ng/mL or more), the effect of FGF-2 on neural progenitor cells is proliferative (Ray et al.,

1993), while epidermal growth factor (EGF) only acts on cell proliferation and stem cell renewal (Kuhn et al., 1997). Other intrinsic factors, such as cell cycle regulators, transcription factors and epigenetic factors, also play important roles in regulating neurogenesis. [for detailed reviews, (Ming and Song, 2011; Zhao et al., 2008b)]. While there are some commonalities, SVZ and SGL are considered to be two different niches with molecular signals unique to each niche.

Environmental enrichment and physical exercise have both been shown to effectively promote cell proliferation and survival of adult neural stem/progenitor cells. Voluntary or forced running on treadmill both robustly increases neural progenitor cell proliferation in the hippocampus, but not in the SVZ (Brown et al., 2003; Farmer et al., 2004; van Praag et al., 1999b), while enriched odor exposure has been shown to be a positive stimulator of SVZ neurogenesis and odor memory (Rochefort et al., 2002). Long-term physical exercise induces both proliferative and survival of NPCs in the rodents (Kronenberg et al., 2003). Even more fascinating, it has been shown that the hippocampal neurogenic effect can be passed from parents to offspring (Bick-Sander et al., 2006). On the other hand, cognitive stimulation as observed in EE exhibits prominently neural progenitors survival-promoting effect (Kempermann and Gage, 1999; Kronenberg et al., 2003). These evidence resulted in a current "neural reserve", or so-called "cognitive reserve" postulate, which is a theory that states the ability of the brain to compensate functional loss associated with neurodegeneration and normal aging (Stern, 2002). This implies that continuous experience in EE early in life may provide a compensatory buffer of brain plasticity for the later episodes of neurodegenerative- or age-related dysfunctions, including cognitive loss.

One of the prominent factors that affect adult neurogenesis is aging. The effects of age on neurogenesis was first reported along with the discovery of adult neurogenesis

phenomenon by Altman in 1965, where he showed a decrease in cell genesis within few months after birth (Altman and Das, 1965). More studies were conducted later by several research groups showing the reduced number of proliferating cells with aging in both SVZ and SGL (Barker et al., 2005; Luo et al., 2006; Seki and Arai, 1995). Taken together, this may suggest that while neurogenesis in the adult brain takes place throughout life, its pace is dramatically reduced with aging.

Although there is evidence to suggest that neurogenesis takes place in the human brain (Eriksson et al., 1998), its extent and nature remain elusive, mainly due to the technical difficulties in detecting these processes in the living human brains (Curtis et al., 2007; Hansen et al., 2010; Sanai et al., 2004; Scharfman and Hen, 2007). In this regard, some pioneering studies have been using ^{1}H-MRS techniques for the detection of neurogenesis in the human brain, but further studies are required to gain more insights about how adult human neurogenesis is regulated (Manganas et al., 2007).

E. Neurogenesis and Alzheimer's disease

Recent studies demonstrate that the pathological process in AD might interfere with adult hippocampal neurogenesis (Lovell et al., 2006; Tatebayashi et al., 2003). However, because neurogenesis is known to be dramatically reduced during aging, one should consider the possibility that this reduction may induce neuronal vulnerability in the aging brain, making it more susceptible for neurodegeneration and functional decline. Alterations in SVZ and SGL neurogenesis can very much contribute to cognitive declines and olfaction deficits in AD. In fact, impairment in olfaction is one of the earliest sign of AD. Both of these symptoms are indicative of the significant relationship between neurogenesis and AD. Interestingly, a few molecules related to AD have been shown to play critical roles in regulating adult neurogenesis. Our group has previously demonstrated that knock-down of

PS1 by lentiviral vector expressing PS1-siRNA facilitated neuronal differentiation and decreased proliferation of neural progenitor cells in a γ-secretase-dependent manner (Gadadhar et al., 2011). We also showed that sAPPα is a proliferative factor for EGF-responsive NPCs in the SVZ (Demars et al., 2011). Moreover, knocking out ApoE has also been shown to increase NPCs proliferation in the dentate gyrus (Yang et al., 2011). And in contrast to proliferative effects of sAPPα, APP Intracellular domain (AICD) was previously shown to decrease hippocampal progenitor cell proliferation and survival (Ghosal et al., 2010).

Deficits in neurogenesis have also been reported in many FAD-linked mouse models. Transgenic mice expressing human APP$_{751}$ under mouse Thy-1 promoter showed significant decrease of hippocampal neurogenesis at 3 months of age (Rockenstein et al., 2007). Single mutation of PS1 in PS1$_{M146V}$ knock-in mice showed significantly impaired neurogenesis in SGL that correlated to the decreased performance in contextual fear conditioning tasks at 3 months of age (Wang et al., 2004). Transgenic mice expressing human mutant PS1$_{P117L}$ also showed decreased neuronal survival of NPCs four weeks post-BrdU administration (Wen et al., 2004). Several AD mouse models expressing APP mutants or co-expressing APP and PS1 mutations also demonstrated alteration in neurogenesis. APP/PS1 double knock-in mice showed decreased long-term survival of new neurons at 9 months of age and continuously persisted till 18 months of age (Zhang et al., 2007). In our FAD mouse model, we showed that APPswe/PS1ΔE9 mice exhibit impaired cell proliferation and differentiation as early as 2 months of age, before the onset of amyloid deposition (Demars et al., 2010), as well as impaired neuronal survival in the hippocampus (Verret et al., 2007). In a separate study, 3xTg-AD mouse model of AD also showed age-dependent decrease in cell proliferation that directly associated with the extent of amyloid

deposition (Rodriguez et al., 2008). However, in spite of the majority of evidence for compromised neurogenesis in many AD mouse models, controversial observations have also been reported showing an increase in neuronal proliferation and differentiation in J20 strain APPswe, Ind mice and in mice with PS1$_{A264E}$ mutation (Chevallier et al., 2005; Jin et al., 2004a; Lopez-Toledano and Shelanski, 2007). All of the above studies suggest that alteration in neurogenesis in FAD mouse models is greatly associated with the intrinsic effects of FAD mutations and AD pathology. In addition, alterations in hippocampal neurogenesis, cell cycle regulation and neurogenesis markers in AD patients have also been reported in several studies (Boekhoorn et al., 2006; Lovell et al., 2006; Lovell and Markesbery, 2005). Knowing how to modulate neurogenesis in AD may provide a potential therapeutic strategy to prevent AD or slow down the progress of the disease. More research studies are required to gain better understanding of the temporal and spatial pattern and the roles of neurogenesis in AD.

F. The environmental enrichment (EE) paradigm

Environmental enrichment (EE) is a term for exposing laboratory animals to a combination of complex environment that facilitates enhanced sensory, cognitive and motor stimulation (Nithianantharajah and Hannan, 2006). The experimental concept of environment-induced plasticity was adapted from Donald Hebb's original theory of "use-dependent plasticity", where he demonstrated that a combination of complex stimuli and social interaction during critical period enhanced the development and learning in young rodents, and the effect could persist into adulthood (Hebb, 1947, 1949). Later in the 1960s, two research groups initiated the studies of the effect of environmental stimuli on brain structure and function. Research lead by Hubel and Wiesel showed that early visual

deprivation during development in the kittens affected the anatomy and physiology of the visual cortex (Wiesel and Hubel, 1965).

Rosenzweig's group was the first group to show that EE resulted in the increased weights and cortical thickness of the rat brains. They further established the effects of EE on anatomical, biochemical, developmental and behavioral changes in the rat brain, and these studies have formed the experimental basis for EE paradigms that we use today (Rosenzweig and Bennett, 1972; Rosenzweig et al., 1978; Rosenzweig et al., 1964; Rosenzweig et al., 1962; Rosenzweig et al., 1968). Since then, numerous studies have focused on the effect of EE on CNS in healthy and brain-damaged animals, further demonstrated additional beneficial effects of EE, such as increased number of and dendritic spines synapses [(Ip et al., 2002), for review see (Rosenzweig and Bennett, 1996)], enhanced extent of branching per neuron, as well as enhanced packing density of synaptic vesicles in the synapses (Greenough and Volkmar, 1973; Greenough et al., 1973; Nakamura et al., 1999; Turner and Greenough, 1985; West and Greenough, 1972).

Consequently, numerous reports showed that EE consists of stimuli that elicits a board spectrum of plasticity changes in the adult brain, ranging from anatomical and neurochemical changes to neurogenesis, gliogenesis, synaptogenesis and cognitive improvement [reviewed in (van Praag et al., 2000)]. Functionally, EE enhances memory function in various learning tasks, such as maze learning (Wainwright et al., 1993), spatial memory (Fordyce and Wehner, 1993; Pacteau et al., 1989), working memory (van Praag et al., 1999a), and problem solving (Renner and Rosenzweig, 1987). In the long-term, EE can also induce changes in gene expression profiles in specific brain regions, particularly those that are related to learning and memory, such as in the hippocampus (Keyvani et al., 2004; Rampon et al., 2000; Thiriet et al., 2008). It should be noted that the extent of response to

EE, either physiologically or behaviorally, may depend on the age of the animals, time and duration of experience in EE, gender, composition of EE and experimental setups.

G. Effects of environmental enrichment on neurogenesis and AD

Probably the first evidence suggesting that environmental complexity may enhance postnatal neurogenesis was provided by a study from Barnea and Nottebohm in 1994, where they found more new hippocampal neurons in the brain of wild free-ranging adult black-capped chickadees, compared to those living in captivity (Barnea and Nottebohm, 1994). The extent of hippocampal new neurons was well-correlated with natural variation in food-storing behavior, a routine behavior that involved learning and spatial memory in these birds (Clayton, 1998). In mammalian studies, Kempermann and his colleagues were the first to show that there were more hippocampal neurons in adult mice living in an enriched environment (Kempermann et al., 1997). Several later studies further showed that experience in an enriched environment increased the survival of newborn cells in the dentate gyrus of these mice (Kempermann and Gage, 1999; Kempermann et al., 1998b; Pencea et al., 2001b), as well as it enhanced their spatial memory functions (Frick and Fernandez, 2003; Frick et al., 2003; Pacteau et al., 1989). In addition, voluntary physical activity alone was also reported to increase cell proliferation and neurogenesis in the dentate gyrus of adult mice (van Praag et al., 1999b), in addition to enhanced spatial learning in rodents (Fordyce and Farrar, 1991; Fordyce and Wehner, 1993; van Praag et al., 1999a). Importantly, not only did it improve spatial memory in young adult mice, EE was also found to improve spatial memory and cognitive function in aged rodents, indicating the contribution of EE to the construction of cognitive reserve (Bennett et al., 2006). Importantly, this may suggest that while brain plasticity and function decline with age, they can be upregulated once the appropriate stimuli are provided.

Since the majority of AD cases are sporadic, late-onset, it is logical to assume that environmental factors play an important role in the formation and/or progression of the disease. Numerous studies during the past few years examined the effects of EE and/or voluntary exercise on neuropathology of AD, neuroplasticity and cognitive performances. The examination of the effect of EE on Aβ levels was proven to be quite complex and current data present some controversial results. Using transgenic mice harboring APPswe/PS1ΔE9, Lazarov and colleagues have previously shown that enriched environment significantly reduced the level of amyloid deposition (Lazarov et al., 2005b). Similar observations were reported in other EE studies using several different FAD-linked transgenic mouse models, where many studies reported reduction of Aβ plaques (Adlard et al., 2005b; Ambrée et al., 2006; Cracchiolo et al., 2007), reduced Aβ*56 associated with cognitive decline (Billings et al., 2007), or reduce Aβ1-42/ Aβ1-40 ratio (Mirochnic et al., 2009). However, other studies have shown no change (Arendash et al., 2004; Cotel et al., 2012; Richter et al., 2008; Wolf et al., 2006) or even increase in Aβ levels (Jankowsky et al., 2005; Jankowsky et al., 2003). Interestingly, one study reports the reduction in Aβ level in enriched PDAPP mice only in those that undergo behavioral testing, but not to those that experienced in EE alone (Costa et al., 2007). In this current study, we have shown that EE significantly reduces the level of oligomeric Aβ levels in APPswe/PS1ΔE9 mice (Hu et al., 2010). Many of these controversial findings can be explained by the use of different experimental designs, age of the animals, genders and other factors critical for the extent of amyloidosis. In regard to NFTs, none of the studies so far has reported the effect of EE on tau hyperphosphorylation. One study has evaluated the effect of behavioral trainings on the extent of tau phosphorylation, where they showed significant reduction of phosphorylated tau level as detected by AT8, AT180 and AT270 antibodies after learning (Billings et al.,

2007). In fact, our study is the first to study the effect of EE on tau hyperphosphorylation. Similar to the effect of learning in 3xTg-AD model, we showed that EE can effectively reduce levels of tau hyperphosphorylation in APPswe/PS1ΔE9 mice (Hu et al., 2010).

Evidence from several human and animal studies suggests that there is a lack of direct correlation between the extent of amyloid deposition and cognitive performances (Josephs et al., 2008; Sloane et al., 1997; Vemuri et al., 2011; Villemagne et al., 2011), suggesting that improvement in cognitive functions following EE may or may not depend on changes in Aβ levels. In fact, the extent of NFTs is better correlated with cognitive declines (Ghoshal et al., 2002). Although the effect of EE on amyloid metabolism in animal studies is debatable, the consensus seems to be that the experience of FAD-linked transgenic mice in an enriched environment enhances learning and memory in various cognitive tasks (Adlard et al., 2005b; Ambrée et al., 2006; Arendash et al., 2004; Billings et al., 2007; Costa et al., 2007; Cracchiolo et al., 2007; Jankowsky et al., 2005; Jeong et al., 2011; Wolf et al., 2006). With the exception of one study, where it showed no changes in all categories tested, using APPswe/PS1KI mouse model experienced in EE (Cotel et al., 2012). Although a few studies showed that physical exercise alone is adequate to ameliorate amyloid pathology and improve cognitive deficits (Adlard et al., 2005b; Nichol et al., 2007; Nichol et al., 2008), many of these studies also suggest that running alone is not sufficient to correct AD-related cognitive deficits in these mice and that full EE setup offers a better overall outcomes (Cracchiolo et al., 2007; Richter et al., 2008; Wolf et al., 2006). It must also take into consideration that depending on the transgenic models of AD, the effects of EE and/or physical exercise on AD pathology and cognitive functions can be varied to different extents.

Increasing evidence suggests that altered or compromised neurogenesis may contribute to the cognitive impairments and neuronal vulnerability that are characterized in

AD [for review see (Lazarov and Marr, 2010)]. Indeed, numerous studies report impaired hippocampal neurogenesis in mouse models exhibiting high level of Aβ and/or amyloid deposition (Demars et al., 2010; Ermini et al., 2008; Niidome et al., 2008; Taniuchi et al., 2007; Verret et al., 2007; Zhang et al., 2007), and neurofibrillary tangles (Rodriguez et al., 2008). Because the hippocampus is one of the earliest areas in the brain to be affected in AD, it is reasonable to assume that AD pathology may affect and alter neurogenesis early in the disease. In fact, we showed that APPswe/PS1ΔE9 transgenic mice exhibit impairments in hippocampal neurogenesis early in life, at 2 month of age (Demars et al., 2010). This observation is in agreement with other studies, suggesting significant impairments in hippocampal neurogenesis in various models of FAD transgenic mice as young as 3 months of age (Knuesel et al., 2009; Wang et al., 2004; Wen et al., 2002) and in older ages (Dong et al., 2004; Donovan et al., 2006; Haughey et al., 2002b; Herring et al., 2009). Of particularly interest is whether EE can rescue impaired neurogenesis and/or promote ongoing adult neurogenesis in AD mouse models. Along with many other studies using various AD mouse models, current results support the notion that EE stimulates neurogenesis in numerous AD models (Catlow et al., 2009; Herring et al., 2009; Hu et al., 2010; Mirochnic et al., 2009; Wolf et al., 2006), and better yet rescue impaired neurogenesis in several studies (Herring et al., 2009; Hu et al., 2010). It is yet to be determined what component in EE underlies the effects on neurogenesis and neuroplasticity. Recent study suggests that it is the running component in EE that is responsible for the neurogenic and neurotrophic effects in wild-type mice (Kobilo et al., 2011). However, it is worth mentioning that many studies reported that running alone is sufficient for the stimulation of neurogenesis in AD mouse models (Catlow et al., 2009;

Mirochnic et al., 2009). **Table I** summarizes the studies that examined the effects of EE and physical exercise on various FAD-linked mouse models.

H. EE and physical exercise: evidence in clinical studies

EE is a very powerful tool for establishing a non-pharmacological and possibly a pharmacological intervention for human disorders. The magnitude of applications for EE is enormous. It ranges from neurodegenerative diseases to psychiatric disorders to cardiovascular diseases, such as strokes. In neurodegenerative diseases and aging, the combination of physical exercise and continuous cognitive stimulation is essential in constructing cognitive reserves, and in prevention or delay of age-related cognitive declines (La Rue, 2010).

Due to the differences in backgrounds and lifestyles, it is somewhat difficult to define what is an enriched environment for each different individuals and to come up with clinical studies to study the effect of EE on aging and cognitive declines in humans. However, there are numerous prospective studies and randomized controlled trials involving humans that investigate the influence of physical exercise in older adults with normal cognition, mild cognitive impairment (MCI) and dementia. Exercise training has been shown to increase size of hippocampus and memory in human clinical studies (Erickson et al., 2011). Still, the evidence in this area is unsatisfactory and it requires more systematic protocols and approaches to make any significant progress (Lautenschlager et al., 2012).

The vast majority of the human studies are epidemiological, observational studies investigating the association between exercise and cognitive functions or dementia in older population that often show inconsistent results. Evidence obtained from meta-analysis of 15 perspective studies by Sofi and colleagues reported a significant protective effect for all levels of physical activity against cognitive declines in older adults with normal cognition

(Sofi et al., 2011). Another systematic review by Plassman and colleagues investigated the evidence provided by over 120 observational studies between 1984 and 2009, each with 300 or more participants and randomized controlled trials with 50 or more participants who were 50 years or older. They concluded a few potentially beneficial factors from the evidence on risk or protective factors associated with cognitive declines, however the authors also commented that the quality of the overall research was quite low and more studies are needed to provide further evidence of the benefits of physical activity (Plassman et al., 2010).

There are several randomized-controlled trials conducted by different research groups to investigate the effect of physical activity interventions in patients with dementia, however, more clinical studies are needed. A study by Willis and colleagues investigated the long-term effects of cognitive training on everyday functional outcomes in elderly. This was a large clinical trial consisted of more than 2,000 participants with five-year follow-up completed in 67% of the sample. They reported an overall significant improvement in performing instrumental activities of daily living in those individual undergo cognitive training sessions, compared to the control groups. Moreover, they showed that cognitive training resulted in improved cognitive abilities specific to the abilities trained that continued for 5 years after the initiation of the intervention (Willis et al., 2006). Another randomized controlled trial led by Lautenschiager and colleagues examined the effect of physical activity in 138 participants over 24 weeks of exercise sessions on cognitive function in older adult at risk for AD, also reported an improvement in cognition over an 18-month fellow-up period (Lautenschlager et al., 2008). In a separate randomized controlled study conducted with AD patients in the nursing homes also found a significant correlation between the improvement in cognitive functions and physical activity (Kemoun et al., 2010). The findings in most of these studies were consistent, where physical activity was shown to be beneficial for

cognitive and functional improvement in elderly or patients with dementia (Heyn et al., 2004; Palleschi et al., 1996).

A recent report by Blankevoort and colleagues reviewed 16 different small randomized controlled trials between 1995-2009 across the globe, and they found that physical activity was beneficial in all stages of dementia. They further found that multi-component interventions (e.g. a combination of endurance, strength and balance) led to larger improvement of physical functions, compared to progressive resistance training alone (Blankevoort et al., 2010). Another meta-analysis reported insufficient evidence to conclude whether or not physical activity programs are beneficial for people with dementia (Forbes et al., 2008).

Currently, there are several ongoing clinical studies attempting to further provide evidence of the effects of physical activity on cognitive function and daily tasks performance (Cyarto et al., 2010; Gillette-Guyonnet et al., 2009; Hill et al., 2009; Vidovich et al., 2011). While undoubtedly EE and/or physical exercise has numerous powerful effects on brain structure and function, in healthy, aging and brain disease, several challenges remain as for the translation of EE from the animal models into a human lifestyle. Further studies are warranted for the adequate translation of EE into a therapeutic approach for human.

TABLE I

THE EFFECTS OF ENVIRONMENTAL ENRICHMENT AND PHYSICAL EXERCISE IN VARIOUS FAD-LINKED MOUSE MODELS

Publication	AD models	Stimulus	Time period for EE	Amyloid plaques	Neurogenesis	Cognitive performance after EE (or RUN)	Other notes
Jankowsky, 2003, 2005	APPswe/PS1ΔE9	EE	2-8.5mo, F	↑	N/A	• ↑ water maze, radial arm water maze	
Lazarov, 2005	APPswe/PS1ΔE9	EE	3wks-6mo, M	↓	N/A	N/A	• ↑ neprilysin activity in EE • ↑ IEGs (Arc, Erg1)
Hu, 2010	APPswe/PS1ΔE9	EE	3wk-2mo, M	↓ oligomeric Aβ	↑	N/A	• ↑ LTP in EE groups • ↑ kinesin-1 expression • ↓ tau hyperphosphorylation
Cracchiolo, 2007	APPswe/PS1m146V	1. SH 2. Toy 3. RUN 4. EE	1.5-9mo.	EE: ↓ Toy: ↔ RUN: ↔	N/A	• Social+EE: ↑ • Social+toy: ↔ • Social+RUN: ↔	• Only Social+EE shows ↑of synaptophysin immunoreactivity in CA1 and CA3
Catlow, 2009	APPswe/PS1m146V	1. EE 2. RUN	1- 9(or 10) mo	N/A	EE & RUN: ↑ (24hr BrdU), ↔ (4 wks BrdU)	N/A	• ↔ calretinin (immature neuron marker)
Cotel, 2012	APPswe/PS1KI	EE	2-6mo, M&F	↔	↔	• ↔ elevated plus maze, Y-maze	• Partial improvement in motor deficit
Adlard, 2005	TgCRND8 (APPswe+Ind)	RUN	Short-term: 6-10wks, F Long-term: 1-6mo, F	↓	N/A	• Long-term: ↑ water maze	• ↔neprilysin or insulin-degrading enzyme levels
Ambree, 2006 and Gortz, 2008	TgCRND8	EE	1-5mo, F	↓	N/A	↑ exploratory behavior, ↓ anxiety-related behavior but could not ameliorate deficits in learning and memory	• ↑ microgliosis in EE • ↔ corticosterone level across housings. • ↓ amyloid angiopathy
Richter, 2008 and	TgCRND8	RUN	2.5-5.5mo	↔	N/A	• ↔ object recognition, Barnes maze	• ↓ stereotypic behaviors in EE • ↔ corticosterone level across housings.

Publication	AD models	Stimulus	Time period for EE	Amyloid plaques	Neurogenesis	Cognitive performance after EE (or RUN)	Other notes
Richter, 2008 and	TgCRND8	RUN	2.5-5.5mo	↔	N/A	• ↔ object recognition, Barnes maze	• ↓ stereotypic behaviors in EE • ↔ corticosterone level across housings.
Herring, 2009	TgCRND8	EE	1-5mo	N/A	↑	N/A	• ↓ neurogenesis in Tg SH compare to Wt (1mo BrdU)
Valero, 2011	APPsw,Ind (J9)	EE	4mo-6mo, F	↔	EE: ↑	• ↑ water maze, platform recognition,	• ↑ number, dendritic length and projection to CA3 region
Wolf, 2006	APP23 (APPswe)	1. EE 2. RUN	2.5-11mo, F	EE: ↔ RUN: ↔	EE: ↑ RUN: ↔	• EE: ↑ water maze • RUN: ↔	• EE: ↑NT3, BDNF • RUN: ↓growth factors • EE&RUN: ↔ neurogenesis in Tg SH compare to Wt (1mo after BrdU).
Mirochnic, 09	APP23	1. EE 2. RUN	Young: 6-7mo Old: 18-19mo	Old EE&RUN: ↔, change in $A\beta 42/A\beta 40$ ratio	EE: ↑in both young and old RUN: only↑ in old group	N/A	• ↑ BrdU+ in APP23 SH compared to Wt SH in young group
Arendash, 2004	APP23	EE	16-20mo	↔	N/A	• ↑ water maze, circular platform, platform recognition, radial arm water maze	
Nichol, 2007, 2008	Tg2576 (16-18 mo.old)	RUN	3 wks	RUN:↓ Abeta40 and soluble fibrillar Aβ	N/A	• RUN improved reference/long-term and working/short-term memory	• Significant impairment in long & short term memory in TG at 17-19 months of age, compared to WT. • Exercise shifts immune response from innate to adaptive/alternative response

Publication	AD models	Stimulus	Time period for EE	Amyloid plaques	Neurogenesis	Cognitive performance after EE (or RUN)	Other note
Jeong, 2011	Tg2576	1. EE 2.EE+stress 3. Stress	3-6mo, 12 mo Female only	↔ in all groups	EE: ↑ Stress: ↓	• EE reduced stress-induced spatial memory deficits	• EE reduced stress-induced tau phosphorylation
Levi, 2007	Human ApoE3, Human ApoE4	EE	3wk-7mo,M	N/A	EE: ↑ in ApoE3, ↓in ApoE4	N/A	• EE induced cell apoptosis in ApoE4 mice
Choi, 2008	1) PS1ΔE9 2) PS1m146V	EE	3wk-2mo, M	N/A	↔	N/A	
Costa, 2007	PS1/PDAPP	EE	1-4.5mo or 5.5 mo	EE + behavioral tested: ↓ Non-tested: ↔	N/A	• ↑ water maze, platform recognition, radial arm water maze	• ↑ transthyresin, NF-KB in EE • ↔ neprilysin in EE • ↓ IEGs (Arc, Erg1, ler2)
Billings, 2007	1) 3XTg-AD 2) PS1-KI	Behavioral testing (learning)	Varies: 2mo, 6mo, 9 mo, 12 mo, 15 mo, 18mo	↓ Fibril Aβ and oligomeric Aβ	N/A	N/A	• ↓ tau hyperphosphorylation

EE: Environmental Enrichment; RUN: Running; SH: Standard Housing; Toy: Toys Only

↔ no change in expression/performance level

↑ increased expression/performance level

↓ decreased expression/performance level

III.   MATERIALS AND METHODS

A. MATERIALS

1.   Chemicals and reagents

All chemicals and reagents used for buffers and solutions were molecular biology grade chemicals purchased from Sigma-Aldrich, Calbiochem, Fisher Scientific, Invitrogen and Promega.

2.   Transgenic animals

All animal experimentations follow the guidelines and protocols approved by the University of Illinois at Chicago Institutional Animal Care and Use Committee (IACUC). Mice used in this study are APPswe/PS1ΔE9 transgenic mice, and the nontransgenic littermates with C57BL/6 background serving as controls. The generation of transgenic mice coexpressing human PS1 encoding ΔE9 mutation and mouse APP encoding human Swedish mutations (K595N, M596L) was described previously (Borchelt et al., 1996; Jankowsky et al., 2001; Lee et al., 1997). Animals were maintained in standard laboratory conditions (14/10 hr light-dark cycle) and with full access to food and water *ad libitum*.

B. METHODS

1. Primary neural stem/progenitor cells culture

Neural stem/progenitor cells (NSCs/NPCs) were isolated from the SVZ of the APPswe/PS1ΔE9 and nontransgenic mice at 2 months of age. Primary cell culture protocols were adapted as previously described in (Gritti et al., 2001). To dissect the forebrain subventricular region, a 2mm-thick coronal section was cut initially about 1mm behind the olfactory bulb and subsequently removing the section by cutting 2 mm dorsal to the initial

cut. With a dissecting microscope, the lateral and anterior wall of the ventricles containing the SVZ was dissected. SVZ tissue was then chopped into smaller pieces with a sterile scalpel, collected in Hank's balanced salt solution containing 0.005% papain (w/v), 0.001% cystine (w/v), 0.001% DNAse (v/v), and 0.001% EDTA (w/v) and incubated at 37°C for 45 min. After incubation tissue pieces were centrifuged at 1,000xg for 5 min, the tissue was then dissociated by repetitive pipetting using a P200 pipette. Primary cultures were incubated (37°C, 5% CO_2) for 10 days in complete medium [pure water, DMEM/F12 (Gibco, Carlsbad, CA), glucose (Sigma, St. Louis, MO), $NaHCO_3$ (Sigma), HEPES (Sigma), L-glutamine (Gibco), penicillin/streptomycin (Gibco), putrescine (Sigma), apo-transferrin (Sigma), insulin (Roche, Indianapolis, IN), sodium selenium (Sigma), progesterone (Sigma), bovine serum albumin (BSA; Sigma), heparin (Sigma), epidermal growth factor (EGF; final conc. 20 ng/mL; Peprotech, Rocky Hill, NJ), and basic fibroblast growth factor (bFGF; final conc.10 ng/mL; Peprotech)] before the first passage.

To subculture the cells, NSCs were collected in 15mL conical tubes and pelleted by centrifugation at 1,000xg for 10 min. Old medium was removed and cells were mechanically dissociated with P200 pipette until they are singly dissociated. Singly dissociated cells were then plated at a density of 1×10^4 cells/cm^2 in new medium. Cells were fed with new medium every 4-5 days.

2. *In vitro* 5-Bromo-2'-deoxyuridine (BrdU) proliferation assay

NPCs were dissociated into single cells and were plated into a 96-well plate (15,000 cells/well). Each well was treated with 5 μM BrdU and incubated for 48 hrs at 37°C, 5% CO_2. Cells were then fixed with 70% ethanol/ 0.1 N NaOH for 30 min at room temperature and incubated in mouse mAb BrdU (1:300; Novocastra) for 1 hr at room temperature. Cells were rinsed three times in 1×TBS and incubated in secondary antibody rabbit anti-mouse HRP

(1:5,000; Pierce) for 30 min at room temperature, followed by the addition of tetramethylbenzidine (TMB) substrate solution (Invitrogen) in the dark for 15 min. The reaction was terminated by adding 2.5 N sulfuric acid to each well. Absorbance was measured using a spectrophotometric plate reader at dual wavelengths of 450-595 nm. Each experimental group includes five replicates (N=5).

3. Environmental enrichment

3-wk-old male APPswe/PS1∆E9 mice and their nontransgenic littermates were exposed to environmental enrichment for a period of 1 month (or 2 months of long-term cell survival studies). Mice were maintained in groups of 3-5 males/cage. The enriched environment was composed of running wheels, colors tunnels, visual stimulating toys, and free access to food and water in the enlarged cages (approximately 24×17×11 inches in dimensions), where objects in the cage were changed and repositioned for novel stimulation every day. Mice were exposed to environmental enrichment for 3 hr per day and they were returned to the standard housing cage (approximately 11×6×8 inches in dimensions) for the rest of the day. Control groups of APPswe/PS1∆E9 mice and their nontransgenic littermates were singly housed in a standard laboratory condition for 1 month (or 2 months for the long-term survival study). **Figure 2** shows the experimental setup and a typical arrangement of an enriched environment cage.

4. 5'-Bromo-2'-deoxyuridine (BrdU) administration

A solution of BrdU was prepared at 20mg/mL in sterile saline solution and injected intraperitoneally (i.p.) at a dose of 100mg/Kg every 12 hr for 3 consecutive days on P58 to P60 of environmental enrichment or standard housing. Mice brains were collected 3 hr or 1 month after the last BrdU injection. Experimental timeline was described in **Figure 2**.
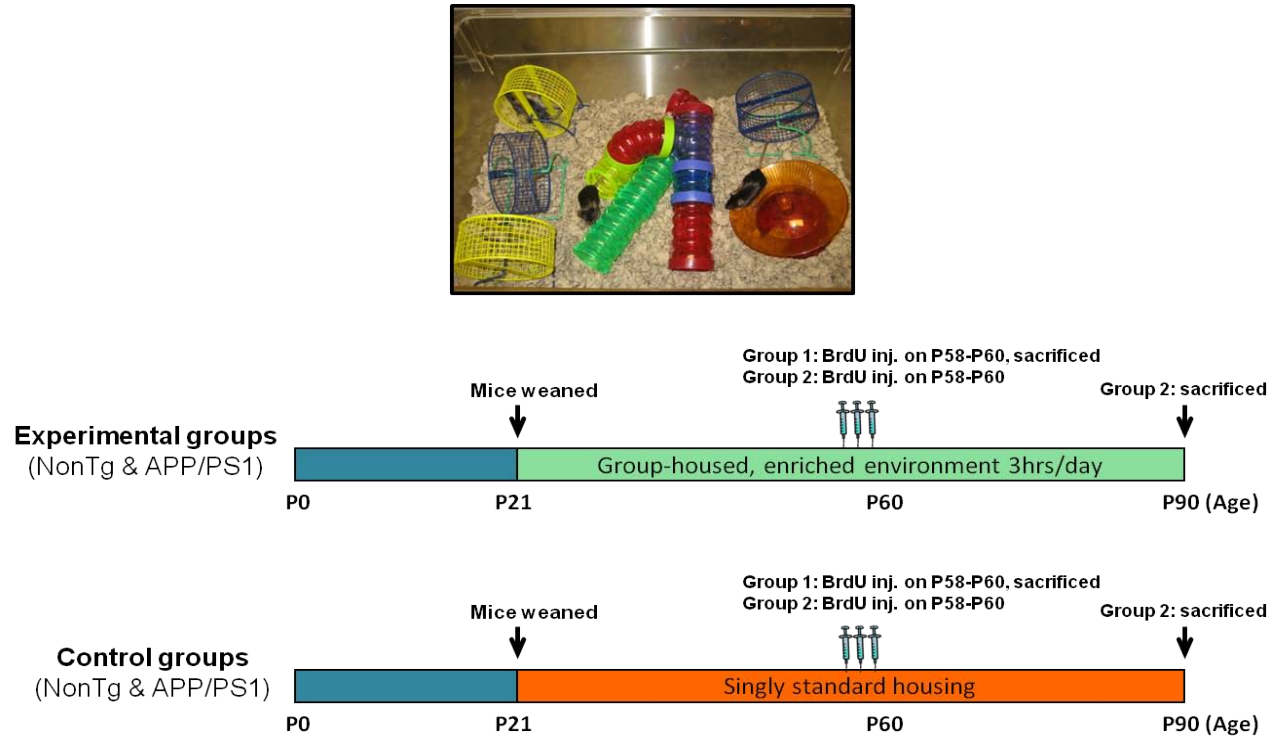
FIGURE 2 Environmental enrichment

(Top) Enrichment cage setup. The photo represents a typical enrichment cage setup in this study. 3-5 male mice from the same litter were grouped at the age of weaning, and the equal number of running wheels was provided. In addition, cages contained colorful toys and tunnels for visual stimulation. The running wheels and toys were rearranged daily to ensure novel environmental conditions. **(Bottom) Experimental design**. Control mice were singly housed in standard laboratory cages. Mice were weaned at P21 and housed 3 to 5 animals per cage (experimental group). Mice in the experimental group experienced an enriched environmental conditions for 3 hrs every day. To examine cell proliferation and early neuronal differentiation in the subgranular layer (SGL), mice were injected with BrdU (dose=100mg/Kg) on the last 3 days of enrichment (P58-P60) and sacrificed 3 hrs after the last BrdU injection (experimental group 1). To examine neuronal survival in the SGL, animals were allowed to experience an enriched environment for another one month and sacrificed at P90 (experimental group 2). Mice in the control groups were singly housed in standard laboratory cages and were subjected to the same BrdU regimen. Abbreviations: Nontg, Nontransgenic; APP/PS1, APPswe/PS1ΔE9

5. **Tissue processing and collection**

Mice were anesthetized with ketamine (100mg/Kg) and xylazine (5mg/Kg) and transcardially perfused with cold 1×PBS. The whole brain was dissected into two hemispheres, one of which was further dissected into different brain regions (olfactory bulb, hippocampus, cortex, lateral ventricle and cerebellum), froze immediately in liquid nitrogen and stored in -80°C for biochemical analysis. For brain tissues used for analyzing kinase expression level, brains were dissected into different regions and froze immediately in liquid nitrogen without perfusion with PBS. The other hemisphere was incubated in 4% paraformaldehyde for 3 days at 4°C and transferred to 30% sucrose solution until the brain reached saturation. For immunohistochemistry, brains were sectioned sagittally into 50 μm thickness with microtome, and each section was stored separately in a 96-well plate immersed in cryoprotectant (glycerin and ethylene glycol in 1×PBS).

6. **BrdU pretreatment**

For BrdU immunohistochemistry, brain sections were pretreated with deionized formamide and antigen retrieval solution (3M sodium chloride, 300mM sodium citrate dihydrate, pH 7.0) for 2 hr at 65°C. Floating sections were then incubated in 2N HCl for 30 min at room temperature, and rinsed in 0.1 M borate buffer for 10 min at room temperature. It was then further proceeded to steps in immunohistochemistry.

7. **Immunohistochemistry**

Brain sections were blocked with normal donkey serum and were subsequently incubated in primary antibody cocktails that consisted of mAb rat anti-BrdU (1:500; Accurate Chemical & Scientific Corporation), biotinylated pAb rabbit anti-glial fibrillary acidic protein (GFAP; 1:500; Dako-Cytomation), pAb goat anti-doublecortin (DCX; 1:400; Santa Cruz

Biotechnology), mAb mouse anti-NeuN (1:400; Chemicon), mAb mouse anti-nestin (1:400; Millipore) and pAb goat anti-sox 2 (1:250; Santa Cruz Biotechnology) for 72 h at 4°C. Immunofluorescence secondary antibodies used in this study were Cy^{TM}3 donkey anti-rat (1:500; Jackson ImmunoResearch), Cy^{TM}5 donkey anti-goat (1:250; Jackson ImmunoResearch), Cy^{TM}5 donkey anti-mouse (1:250; Jackson ImmunoResearch), and Cy^{TM}2-conjugated streptavidin (1:250; Jackson ImmunoResearch). For detection of amyloid plaques, sections were incubated in mAb anti-amyloid beta (3D6) (1:1000; Gift from Dr. Samgram Sisodia, University of Chicago) and visualized with secondary Cy^{TM}3 donkey anti-mouse (1:500; Jackson ImmunoResearch) antibody.

8. <u>Cell counting by stereological analysis</u>

Cell counts of positive cell markers within the dentate gyrus of the hippocampus was performed with design-based stereology (StereoInvestigator version 8, MBF Bioscience), using optical fractionator applying $N_v \times V_{Ref}$ method as described previously (Peterson, 1999; West et al., 1991). For the analysis of total BrdU+, DCX+ and GFAP+ cells, every sixth section was taken for immunohistochemical and stereological analysis. The dentate gyrus was traced at low (10 ×) magnification and all cell counts were performed at high (63 ×) magnification (Zeiss AX10 microscope; Carl Zeiss Ltd.). The sampling parameters were as the following: Counting frame width (X) = 100μm, counting frame height (Y) = 100μm, sampling grid size (X) = 148μm, sampling grid size (Y) = 210μm, dissector height (Z) = 20μm and section periodicity = 6. For the analysis of NeuN+ and S100β+ cells, every third section was taken for immunohistochemical and stereological analysis. The section periodicity was changed to 3, with sampling grid sizes (X and Y) = 100μm, and the counting frame size (X and Y) = 100μm.

9. Two-step protein extraction

Two-step protein extraction was performed to obtain water-soluble fractions for oligomeric Aβ detection and detergent soluble fractions for western blot analysis. First, the tissues from cortex and hippocampus were thoroughly homogenized in phosphate buffered saline (1 ×PBS) containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) and ultracentrifuged at 100,000×g for 1 hr at 4°C as described previously (Kayed et al., 2003). The supernatant was collected and protein concentration was calculated with BCA method (Pierce, Rockford, IL, USA). The pellet was immediately undergo second step extraction in ROLB buffer (10mM HEPES, pH7.4, 0.5% Triton X-100, 80mM β-glycerophosphate, 50mM sodium fluoride, 2mM sodium orthovanadate (Calbiochem), 100nM staurosporine (Calbiochem) , 100nM K252a (Calbiochem,) , 50nM okadaic acid (Calbiochem), 50nM microcystin (Calbiochem), mammalian protease inhibitor cocktail (Sigma-Aldrich), phosphatase inhibitor cocktail II (Calbiochem). Protein quantification was performed using BCA method (Pierce) and equal concentration of proteins was used for immunoblotting experiments.

10. Detection of soluble oligomeric Aβ by Dot-blot Analysis

50µg of water-soluble protein fraction was spotted directly onto the prewetted nitrocellulose membrane assembled within the dot-blot apparatus (Bio-Rad) with vacuum. The membrane was then blocked with 5% non-fat milk/TBST solution for 2 hours at room temperature and incubated in primary antibody, pAb rabbit anti-amyloid oligomer (A11) (1:5000 Millipore) for overnight at 4°C. Next day, the membrane was then incubated in secondary antibody, donkey anti-rabbit HRP (1:20,000, Promega) for 1 hour and the immunoreactivity was detected using ECL^{TM} Plus chemiluminescent substrate (GE

Healthcare). Protein expression levels were quantified by densitometric analysis using ImageJ1.41o software (National Institutes of Health, Bethesda, MD, USA).

11. <u>SDS-PAGE and Western blot Analysis</u>

30 µg of detergent soluble protein samples were separated on 7.5% and/or 12.5% acrylamide gels and were transferred to 0.45 µm nitrocellulose membrane (Bio-Rad) for 2 hours at 100mV in transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3). Membrane blots were blocked for 2 hours in blocking solution (1% BSA in TBS) at room temperature and following by incubation of primary antibodies diluted in blocking solution overnight at 4°C. Next day, the membrane blots were washed three time with TBST (0.1% Tween-20), and the blots were incubated in secondary antibody diluted in TBST for 1 hour. Membranes were visualized with ECL^{TM} Plus chemiluminescent substrate (GE Healthcare), and the protein expression levels were quantified by densitometric analysis using ImageJ1.41o software (National Institutes of Health, Bethesda, MD, USA).

Primary antibodies used in this study were pAb rabbit anti-APP369 (1:2000, generous gift from Dr. Sangram S. Sisodia, University of Chicago), monoclonal mouse anti-phosphorylated Tau (PHF-1 (Otvos et al., 1994), 1:2500, gift from Gustavo Pigino, University of Illinois at Chicago), mAb mouse anti-Tau5 (1: 10,000, Chemicon), mAb mouse anti-Kinesin Heavy Chain (H2) (1:2000, (Pigino et al., 2009)), mAb mouse anti-Kinesin Light Chain (63-90) (1:2000, (Stenoien and Brady, 1997)), mAb mouse anti-Tau5 (1: 10,000, Chemicon), mAb rabbit anti-phospho GSK3β serine 9 (1:2000, Cell Signaling), mAb mouse anti-GSK3β (1:2500, BD transduction), pAb rabbit anti-Akt (1:2000, Cell Signaling), pAb rabbit anti-phospho Akt Serine 437 (1:2000, Cell Signaling), mAb mouse anti-Casein kinase IIα (1:1000, Calbiochem), mAb mouse anti-Cdk5 (Santa Cruz) and mAb mouse anti-Actin (1:2500, Millipore). Secondary horse peroxidase antibodies used in this study were rabbit

anti-mouse HRP (1:5000, Pierce) and donkey anti-rabbit HRP (1:20,000, Promega). Densitometric analysis was analyzed using ImageJ software, normalizing the expression of the protein of interest to its loading control.

12. <u>LTP electrophysiology</u>

Mice (APPswe/PS1ΔE9 and NonTg) were sacrificed within 5 d after the termination of 1 month environmental enrichment, and hippocampal slices were prepared as described (59). Slices were maintained at $35 \pm 1°C$ in an interface chamber constantly perfused (1.0 ml/min) with medium containing 124 mM NaCl, 3 mM KCl, 1.2 mM KH_2PO_4, 26 mMNaHCO$_3$, 10 mM d-glucose, 2.5 mM $CaCl_2$, 2.5 mM $MgSO_4$, and 2 mM Na-ascorbate, gassed with 95% O_2/ 5% CO_2. Schaffer/commissural synapses were stimulated with twisted, bipolar electrodes placed in stratum radiatum (SR) of field CA1a or CA1c; field excitatory postsynaptic potentials (fEPSPs) were recorded with a glass micropipette placed in SR of CA1b. Basal synaptic transmission was assessed by constructing input-output curves using stimulation currents of 2.5–160 μA. Paired-pulse facilitation was assessed with interpulse intervals from 50 to 800 ms. LTP was induced by θ-burst stimulation (TBS) consisting of 2, 4, or 8 high frequency bursts (100 Hz, 4 pulses) repeated at 200-ms intervals (5 Hz). The fEPSP slope was monitored at 20-s intervals for ≥10 min before and 60 min after TBS. LTP was assessed as the potentiation present 30 and 60 min after TBS.

13. <u>BDNF ELISA</u>

Cortex and hippocampus samples from six experimental groups (singly-housed nontransgenic, singled-housed APPswe/PS1ΔE9, group-housed nontransgenic, group-housed APPswe/PS1ΔE9, enriched nontransgenic and enriched APPswe/PS1ΔE9, N=6 per group, total of 72 samples) have been previously collected and store in -80°C. The expression level of BDNF was measured using ELISA kit, BDNF E_{max} ImmunoAssay

System (Promega, WI, USA), according to the manufacturer's instructions. Brain tissues were homogenized in modified protein extraction buffer as described in Szapacs, et al., 2004 and followed by BCA quantification assay to determine the protein concentrations. Briefly, 96-well immunoplates were coated with 100 μL per well of monoclonal anti-mouse-BDNF antibody. After an overnight incubation at 4°C, the plates were washed three times with wash buffer and the samples (at 100 μL each) were incubated in the coated wells for 2 hr at room temperature with shaking. After an additional five washes, the immobilized antigen was incubated with an anti-human BDNF antibody for 2 hr at room temperature with shaking. The plates were washed with wash buffer, and then incubated with an anti-IgY HRP for 1 hr at room temperature. After another wash, the plates were incubated with a TMB/peroxidase substrate solution for 15 min and then 1M HCl (100 μL /well) was added to the wells. The colorimetric reaction product was measured at 450 nm using a microplate reader. BDNF concentration was determined from the regression line for the BDNF standards (range = 7.8–500 pg/ml purified mouse BDNF), incubated under similar condition in each assay. The sensitivity of the assay is about 15 pg/g of BDNF and cross-reactivity with other related neurotrophic factors (NGF, Neurotrophin-3 and Neurotrophin-4) is <3%. All samples were analyzed in duplicate.

14. RNA extraction

Hippocampus were dissected and immediately froze in liquid nitrogen and stored at -80°C. Total RNA was isolated from tissue using RNAeasy Mini Kit (Qiagen), according to the manufacturer's instruction. Briefly, about 20mg of tissue was manually homogenized in 500uL of buffer RLT extraction buffer. The homogenate was centrifuged for 3 minute at full speed, and the supernatant was transferred to new tube. Second centrifugation was performed to remove any remaining cellular debris, following by adding 95% ethanol for

RNA precipitation and binding onto the column. The column was centrifuged and washed several time and finally the RNA was eluted from the column using RNase-free water. RNA concentration was determined using NanoDrop® spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at 260nm/280nm, and RNA integrity was determined by 1% denaturing agarose gel electrophoresis. RNA samples were store at -80°C for further analysis.

15. <u>Reverse transcription and quantitative polymerase chain reaction (qPCR)</u>

cDNA synthesis was performed with SuperScript®III First-strand synthesis SuperMix (Invitrogen), using 1μg of total RNA and oligo dT primers. cDNA was further diluted in deionized water and store at -20°C. The lists of primer sequences and references are listed in **Table II**. All primers were tested with conventional PCR reactions and the efficiency of each primer pairs was tested using standard curve assay.

PCR cycling conditions were: 10 min at 95°C, followed by 40 cycles at 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec. Samples were analyzed in triplicate and a melting curve analysis was performed in each sample at the end of qPCR. Expression level of each gene was determined by BioRad iQ5 icycler real time PCR system employing iQ SYBR Green Supermix (BioRad). The 18S rRNA and GAPDH gene expressions were used as internal controls. Relative gene expression was determined by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The Ct value was determined for the target genes and for the endogenous internal controls in each sample. The difference between target gene and internal control Ct was determined for each sample, resulting in the ΔCt value. The ΔCt of a calibrator sample was subtracted from each sample ΔCt resulting in the $\Delta\Delta$Ct value. Relative fold change was calculated as $2^{-\Delta\Delta Ct}$.

16. <u>Statistical analysis</u>

All data are presented as mean ± SE, unless indicated otherwise. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). Stereological analysis data was analyzed using two-way analysis of variance (ANOVA), following by Bonferroni's post hoc tests. All biochemical analyses (ELISA, densitometry, RT-PCR) were analyzed using Student's t test or one-way ANOVA, following by Tukey's post hoc test. Electrophysiological data were analyzed by repeated measures ANOVA, with genotype and environmental condition as between-subjects factors and stimulus intensity (input-output curves) or interpulse interval (paired-pulse facilitation) as within-subjects factors. LTP data was analyzed using factorial ANOVA with genotype, environmental condition, and TBS burst number as between-subjects factors. All results were considered statistically significant when $P<0.05$.

TABLE II

REAL-TIME RT-PCR PRIMER SEQUENCES

Targets	Sequences	References
BDNF	Forward 5'-CCA TAA GGA CGC GGA CTT GT-3' Reverse 5'-GAG GCT CCA AAG GCA CTT GA-3'	(Pang et al., 2006)
CaMKIIα	Forward 5'-ACG AGG GAG TAG ACA GTG GAA GAC-3' Reverse 5'-GCA GGC AGC AGA GTA GTG GAC-3'	(Zhou et al., 2009)
CaMKIV	Forward 5'-AAA TCA GCC TGG TCC TTG AG-3' Reverse 5'-TCT GGT TTG AGG TCA CGA TG-3'	(Ahmed et al., 2007)
CBP	Forward 5'-CAC AGG CAG GAG GCA TGA C-3' Reverse 5'-CAG TTT GAC TAA AGG GTT GTC CAA-3'	(Lopez-Atalaya et al., 2011)
CREB	Forward 5'-GGA ATC TGG AGC AGA CAA CC-3' Reverse 5'-ATA ACG CCA TGG ACC TGG AC-3'	(Ahmed et al., 2007)
GluR1	Forward 5'-GCT TTG TCA CAA CTC ACG GA-3' Reverse 5' CCT TTG GAG AAC TGG GAA CA-3'	(Lopez-Atalaya et al., 2011; Okun et al., 2010)
IGF-1	Forward 5'-CTG GGC TAG GAA CTG TGA GC-3' Reverse 5'-TAA GTG CCG TAT CCC AGA GG-3'	(Wolf et al., 2006)
NGF	Forward 5'-CAG GCA GAA CCG TAC ACA GA-3' Reverse 5'-GTC TGA AGA GGT GGG TGG AG-3'	(Wolf et al., 2006)
NMDAR	Forward 5'- AAG AGG AGT TCA CAG TCA ATG G -3' Reverse 5'- CAG GTC AAC GCA GAA GCC -3'	(Zhou et al., 2009)
NT-3	Forward 5'-TGC AAC GGA CAC AGA GCT AC-3' Reverse 5'-TGC CCA CAT AAT CCT CCA TT-3'	(Wolf et al., 2006)
PKC-γ	Forward 5'-CTC GTT TCT TCA GCA GCA AA-3' Reverse 5'-GTG AAC CAC AAA GCT ACA GAC T-3'	(Yu et al., 2011)
PSD-95	Forward 5'-GGT GAC GAC CCA TCC ATC TTT ATC-3' Reverse 5'-CGG ACA TCC ACT TCA TTG ACA AAC-3'	(Zhu et al., 2011)
tPA	Forward 5'-TGT CTT TAA GGC AGG GAA GT-3' Reverse 5'-GTC ACA CCT TTC CCA ACA TA-3'	(Sartori et al., 2011)
TrkB	Forward 5-'CGC CCT GTG AGC TGA ACT CTG-3' Reverse 5'-CTG CTT CTC AGC TGC CTG ACC-3'	(Wang et al., 2010)
Internal Controls		
18S rRNA	Forward 5'-CGG CTA CCA CAT CCA AGG AA-3' Reverse 5'-GCT GGA ATT ACC GCG GCT-3'	(Burbach et al., 2004)
GAPDH	Forward 5'-TGG TCT ACA TGT TCC AGT ATG AC-3' Reverse 5'-TAG ACT CCA CGA CAT ACT CAG C-3'	(Zhou et al., 2009)

All primer sequences were published previously as indicated in the references.

IV. RESULTS

A. Characterization of the effects of FAD-linked mutations on brain plasticity of APPswe/PS∆E9 mice.

1. Proliferation of cells in the SVZ and SGL is significantly reduced in APPswe/PS1∆E9 mice as early as 2 months of age.

Previous studies report impaired hippocampal neurogenesis in several AD mouse models that exhibit high level of amyloid deposition (Demars et al., 2010; Ermini et al., 2008; Niidome et al., 2008; Taniuchi et al., 2007; Verret et al., 2007; Zhang et al., 2007) and neurofibrillary tangles (Rodriguez et al., 2008). Nevertheless, it is not clear whether this is a result of the extensive pathology or an impairment characteristic of the disease. To address that, we examined the extent of neurogenesis in young AD mice (2 months of age), prior to major pathology. We first examined the extent of cell proliferation in the neurogenic regions of transgenic mice co-expressing APPswe/PS1ΔE9 mutations. For this purpose, FAD-linked transgenic mice harboring APPswe/PS1ΔE9 as well as nontransgenic mice were injected with BrdU solution at 2 months of age, 3 months prior to onset of amyloid deposition in APPswe/PS1ΔE9 mice (Jankowsky et al., 2001). Using stereological analysis, we quantified the number of BrdU-positive cells in the SVZ and SGL of these mice (**Figure 3A and Figure 4A**). Stereological analysis of immunolabeled sections revealed that there was a dramatic reduction in cell proliferation, as detected by BrdU, in both the SVZ (**Figure 3B**) and dentate gyrus (**Figure 4B**) of APPswe/PS1ΔE9 mice, when compared with the nontransgenic controls. These results suggest that either the AD mutations intrinsically affect the proliferation of NPC or that the alterations in the neurogenic niche of APPswe/PS1ΔE9 take place long before amyloid plaque formation.

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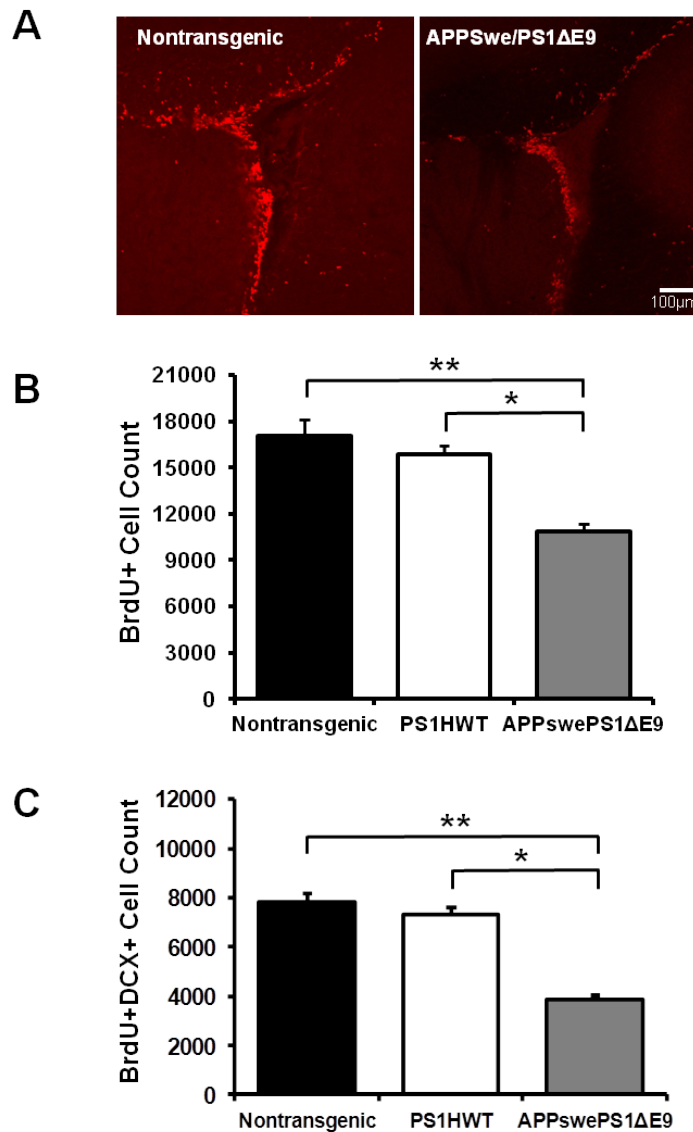
FIGURE 3 Reduced cell proliferation and neuronal differentiation in the subventricular zone of FAD-linked mice co-expressing APPswe/PS1ΔE9 mutations at 2 months of age.

(**A**) Representative images of BrdU+ cells (red) in the subventricular zone (SVZ) of nontransgenic and APPswe/PS1ΔE9 mice. (**B**) The number of BrdU immunoreactive (BrdU+) cells was reduced by 36% in the subventricular zone of mice co-expressing APPswe/PS1ΔE9 mutations when compared with PS1HWT or nontransgenic littermates (*P<0.001, **P<0.05, respectively, Student's t test, N=5 per group). (**C**) The number of BrdU immunoreactive cells that were co-labeled with the early neuronal differentiation marker, doublecortin (BrdU+DCX+), is dramatically reduced in APPswe/PS1ΔE9 mice when compared with PS1HWT or nontransgenic controls.(*P<0.0001,**P<0.001, Student's t-test, N=5 per group). All data was presented as mean ± SE. Scale bar=100μm.

2. <u>APPswe/PS1ΔE9 mice exhibit impaired neuronal differentiation at 2 months of age.</u>

To examine whether expression of FAD-linked mutants also affects the neuronal differentiation of NPCs, brain sections were immunolabeled with antibodies raised against BrdU and the early neuronal differentiation marker doublecortin (DCX). Newly differentiating neurons expressing DCX were quantified in the SVZ and SGL of APPswe/PS1ΔE9 and nontransgenic mice by stereological analysis. We observed a drastic reduction in the number of BrdU+DCX+ cells in both the SVZ (**Figure 3C**) and SGL (**Figure 4C**) of APPswe/PS1ΔE9 mice, suggesting that neuronal differentiation is impaired in these animals. Taken together, these results suggest that cell proliferation and neuronal differentiation of NPCs are impaired in both the SVZ and SGL of APPswe/PS1ΔE9 transgenic mice, and that these impairments take place very early in life, prior to the onset of amyloid deposition.

3. <u>Neurogenic regions of APPswe/PS1ΔE9 mice exhibited high level of tau hyperphosphorylation.</u>

To examine the possibility that alterations in tau phosphorylation take place in the neurogenic niches of APPswe/PS1ΔE9 mice and may underlie impaired neurogenesis, we examined expression levels of tau in the brains of these mice. For this purpose, we prepared protein extracts of the lateral ventricles which consist of SVZ, hippocampus, and a non-neurogenic area (e.g. cortex or cerebellum) from 4-month-old mice and compared tau expression across FAD-linked transgenic mice harboring PS1HWT, PS1ΔE9, and APPswe/PS1ΔE9 using tau phosphorylation specific antibodies. To examine alterations in tau phosphorylation, we used AT8 antibody that detects phosphorylation at Ser-202 and Thr-205 epitopes of tau (Goedert et al., 1995). This phosphorylation-dependent
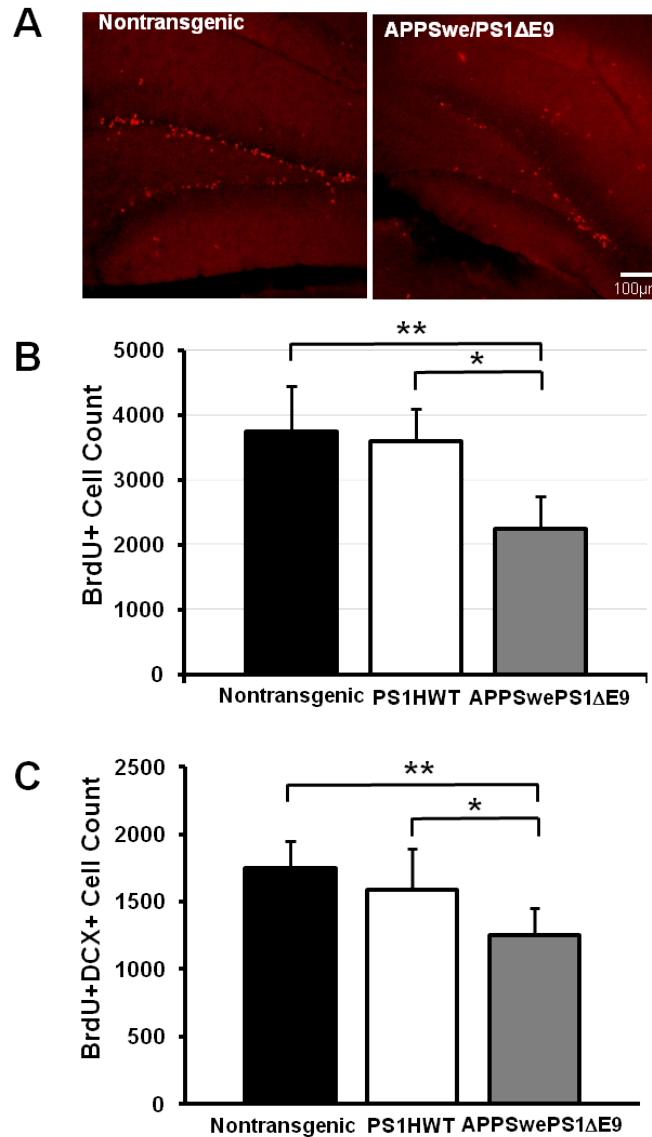
FIGURE 4 Reduced cell proliferation and neuronal differentiation in the subgranular layer of FAD-linked mice co-expressing APPswe/PS1ΔE9 mutations at 2 months of age.

(**A**) Representative images of BrdU immunoreactivity (red) in the subgranular layer (SGL) of nontransgenic and APPswe/PS1ΔE9 mice. (**B**) The number of BrdU immunoreactive (BrdU+) cells was substantially reduced in the SGL of mice co-expressing APPswe/PS1ΔE9 mutations when compared with PS1HWT or nontransgenic littermates (*$P<0.05$, **$P<0.05$, respectively, Student's t test, N=5 per group). (**C**) The number of BrdU immunoreactive cells that were co-labeled with the early neuronal differentiation marker, doublecortin (BrdU+DCX+), was also vastly reduced in APPswe/PS1ΔE9 mice when compared with PS1HWT or nontransgenic controls (*$P<0.05$,**$P<0.01$, Student's t-test, N=5 per group). All data was presented as mean ± SE. Scale bar=100μm.

antibody recognizes hyperphosphorylated tau in both paired helical filaments and NFTs (Biernat et al., 1992; Goedert et al., 1993). Analysis of AT8 levels in the protein extracts revealed a significant increase of phosphorylated tau levels in the SVZ, hippocampus and cortex of APPswe/PS1ΔE9 mice, when compared to equivalent brain regions of PS1ΔE9 and PS1WT mice (**Figure 5A**). These results strongly suggest that the increased level of tau phosphorylation is pronounced in the brain of these mice and in neurogenic niche in particular. To further investigate the alterations in tau phosphorylation, we used PHF-1 monoclonal antibody that recognizes phosphorylated epitopes at Ser-396 and Ser-404 of tau (Mercken et al., 1992; Otvos et al., 1994). The results showed a dramatic increase in PHF-1 expression level in the neurogenic areas and in the SVZ of mutant mice expressing APPswe/PS1ΔE9 and PS1ΔE9, when compared to PS1HWT controls (**Figure 5C, 5D**). Interestingly, in the non-neurogenic regions such as the cortex, the PHF-1 levels in mice expressing APPswe/PS1ΔE9, PS1ΔE9, and PS1HWT were comparable (**Figure 5C**).

Taken together, these results suggest that the significant increase in tau phosphorylation level at the epitopes that identified as clinically relevant in AD occur in the neurogenic areas in the brains of APPswe/PS1ΔE9 mice, and it may underlie impaired neurogenesis. These results further raised the possibility that the increased levels of phosphorylated tau are the result of either altered regulation or altered function of kinases or phosphatases in the neurogenic microenvironments of APPswe/PS1ΔE9 mice.

4. **Neurospheres isolated from SVZ of APPswe/PS1ΔE9 exhibit impaired proliferation and tau hyperphosphorylation.**

Next we examined whether the expression of FAD-linked APPswe/PS1ΔE9 mutations intrinsically affects NPC proliferation. To address this question, we isolated NPCs
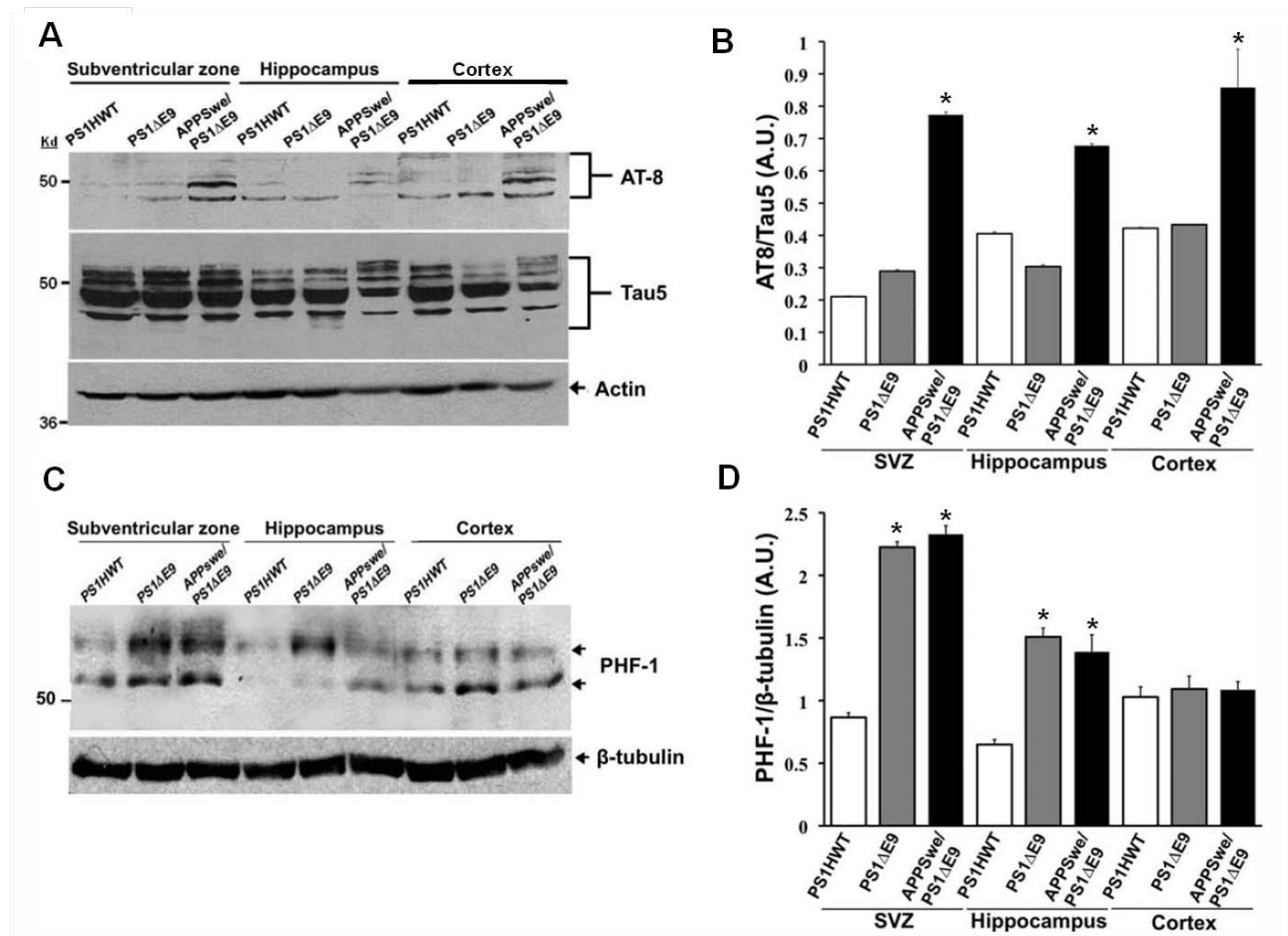
FIGURE 5 Increased levels of phosphorylated tau in the neurogenic regions of APPswe/PS1∆E9 mice.

(**A**) Expression levels of total tau and phosphorylated tau in brain lysates of SVZ, hippocampus, and cortex of PS1HWT, PS1∆E9, and APPswe/PS∆E9 mice, as detected by Western blot analysis using tau-5 and AT-8 antibody, respectively. A dramatic increase in tau phosphorylated at Ser202/Thr205 was detected by AT8 antibodies in all regions of the APPswe/PS∆E9 mice tested. (**B**) Quantification of the amount of AT8/actin relative to tau/actin. (**C**) Levels of phosphorylated tau in brain lysates of SVZ, hippocampus, and cortex of PS1HWT, PS1∆E9, and APPswe/PS1∆E9 mice as detected by PHF-1 antibody. Western blot analysis shows a marked increase in tau phosphorylated at Ser396/Ser404 in the neurogenic regions but not in the cortex. **(D)** Quantification of the relative amount of PHF-1 tau to β-tubulin. All data was presented as mean arbitrary units (A.U.) ± SE. *$P<0.05$, Student's t test, N=5 per group.

from the SVZ of 2-month-old APPswe/PS1ΔE9 and from the SVZ of their nontransgenic littermates, and we examined the extent of *in vitro* proliferation by utilizing BrdU incorporation assay. One unique characteristic of NPCs is that they form neurospheres in culture (**Figure 6A**). We observed a significant reduction in the extent of NPC proliferation isolated from the SVZ of APPswe/PS1ΔE9 transgenic mice compared with neurospheres isolated from the SVZ of their nontransgenic littermates (**Figure 6B**). This suggests that the expression of APPswe/PS1ΔE9 mutations intrinsically impairs proliferation of NPCs, independently of the neurogenic microenvironment. We next examined whether APPswe/PS1ΔE9-expressing NPCs isolated from this area exhibit tau hyperphosphorylation. We extracted protein from neurospheres isolated from the SVZ of APPswe/PS1ΔE9 and nontransgenic mice and examined tau phosphorylation level by Western blot analysis. Intriguingly, we observed that although the total tau levels were comparable in APPswe/PS1ΔE9 and nontransgenic neurospheres, the phosphorylated tau levels as detected by AT8 antibodies were significantly higher in neurospheres derived from the APPswe/PS1ΔE9 mice (**Figure 6C, 6D**). These results suggest that APPswe/PS1ΔE9 mutants induce hyperphosphorylation of tau in NPCs and possibly reduce their proliferative capacity.

B. Environmental enrichment rescues impaired neurogenesis, enhances synaptic plasticity and attenuates neuropathology in FAD-linked APPswe/PS1∆E9 mice.

1. <u>Reduced number of proliferating cells in the SGL of FAD-linked APPswe/PS∆E9 mice can be rescued by experience in environmental enrichment.</u>

To examine the effect of EE on modulating neuroplasticity in FAD-linked APPswe/PS1∆E9 mice, 3-wk-old transgenic and nontransgenic mice were weaned from their parental cages and were exposed to environmental enrichment or maintained in
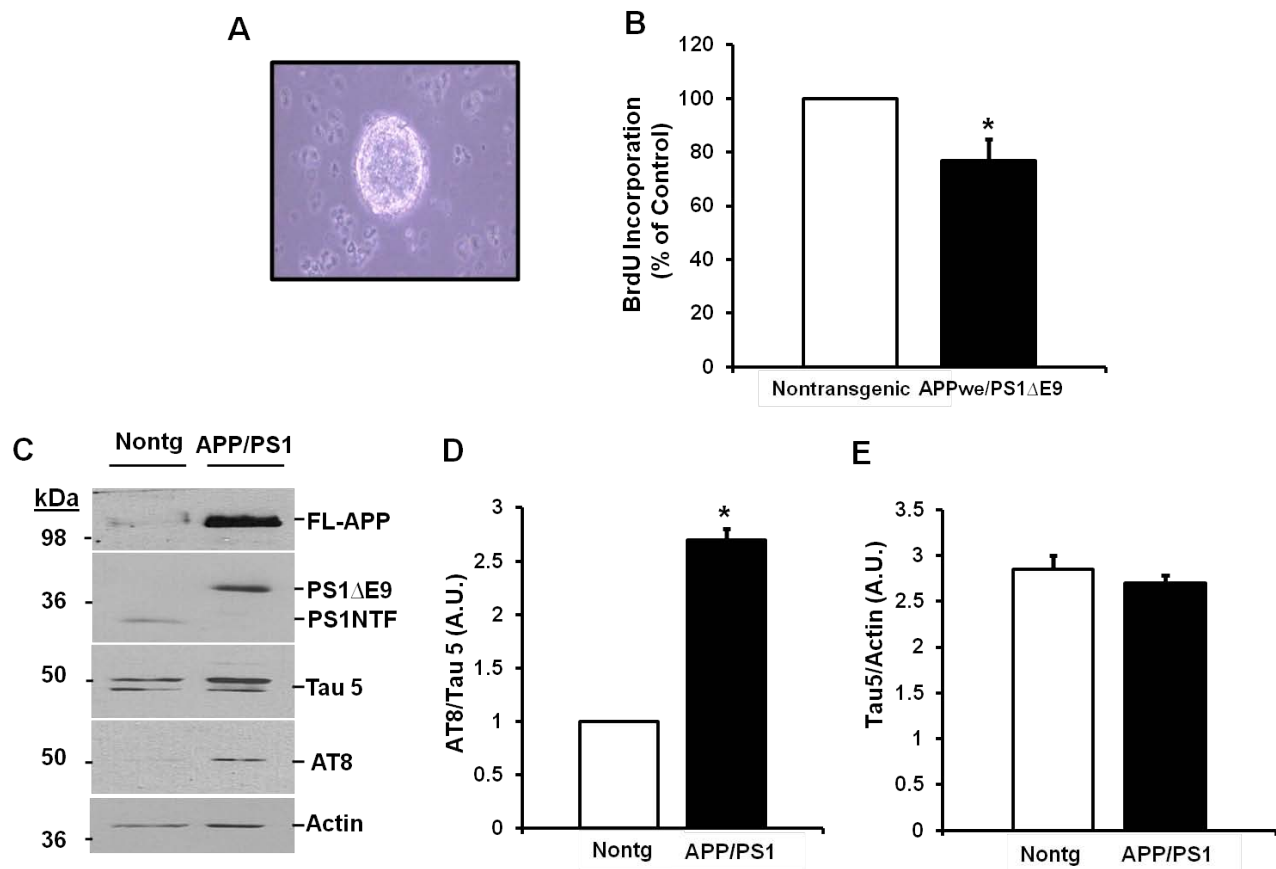
FIGURE 6 Neurospheres derived from the SVZ of APPswe/PS1ΔE9 mice exhibit impaired proliferation and increased tau phosphorylation.

(A) A representative image of a neurosphere *in vitro*. (**B**) *in vitro* proliferation assay examining BrdU incorporation in dissociated neurospheres showed a reduction in the proliferative capacity of neural progenitor cells derived from APPswe/PS1ΔE9 mice compared with neural progenitor cells derived from their nontransgenic littermates. (**C**) Characterization of neurospheres derived from the SVZ of APPswe/PS1ΔE9 mice by Western blot analysis. Neurospheres isolated from APPswe/PS1ΔE9 mice exhibited characteristic transgene expression pattern of APP (two- to three-fold increase in FL-APP) and PS1 (lack of full-length cleavage of PS1ΔE9). While the total tau expression levels were comparable in neurospheres derived from APPswe/PS1ΔE9 and nontransgenic mice, there was a significant increase in level of phosphorylated tau in neurospheres derived APPswe/PS1ΔE9 as detected by AT8 antibody. (**D and E**) Quantification of protein levels as detected by Western blot showed that the level of AT8 was increased three-fold relative to total tau level , whereas the total tau levels are consistent when normalized to actin. Data was presented as mean ± SE (*P<0.05, Student's t-test, N=5 per group).

standard housing conditions for one month. At the end of one month, animals were intraperitoneally injected with BrdU cell proliferation marker every 12 hours for 3 consecutive days. To study the extent of cell proliferation and to characterize the cell lineage in the SGL, brain sections of these mice were immunolabeled with BrdU, glial and neuronal differentiation markers.

As anticipated, the number of BrdU+ cells in the DG of nontransgenic mice that experienced an enriched environmental was significantly increased when compared to the number of BrdU+ cells in the DG of their standard housing littermates, suggesting an increase of cell proliferation following EE (**Figure 7**). We further examined the extent of cell proliferation in the brain of APPswe/PS1ΔE9 mice that experienced an enriched environment. Similar to what we observed with the nontransgenic mice, there was a significant increase of BrdU+ cells in the SGL of APPswe/PS1ΔE9 mice that experienced an enriched environment compared to APPswe/PS1ΔE9 mice housed in a standard laboratory condition. These results indicate that EE promotes cell proliferation in the SGL regardless of the genotypes and disease pathology of the animals.

Another interesting question is whether the ability of NPCs to undergo cellular proliferation is altered due to the expression of the transgenes in APPswe/PS1ΔE9 mice. We applied two-way ANOVA analysis to evaluate whether the extent of increase in the number of proliferating cells in the SGL following EE is consistent across genotypes. The results from two-way ANOVA analysis showed a significant main effect of housing conditions ($F_{1,16}=54.88$, $P<0.0001$) and genotypes ($F_{1,16}=10.84$, $P<0.01$), with no significant interaction between the two parameters ($F_{1,16}=0.08642$, $P=0.7726$), suggesting that the extent of increase in proliferating cells after EE is comparable in the transgenic and nontransgenic mice (**Appendix I, ANOVA Table 1**). Taken together, this suggests that the
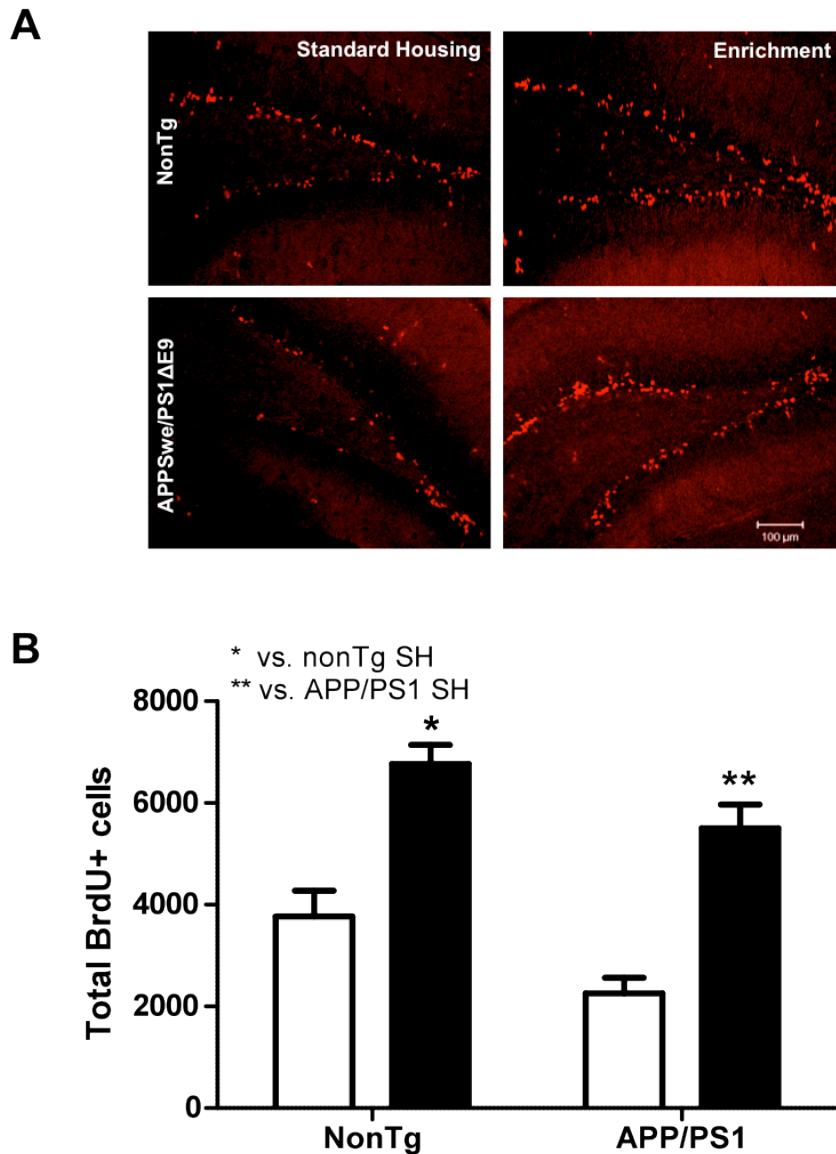
FIGURE 7 Increased cell proliferation in the SGL of APPswe/PS1ΔE9 and nontransgenic mice after experience in an enriched environment.

(**A**) Representative confocal images of BrdU+ cells in the DG of mice maintained in a standard housing and in an enriched environment. (**B**) Total number of BrdU+ cells was significantly increased in APPswe/PS1ΔE9 (APP/PS1) and nontransgenic (NonTg) mice after experience in an enriched environment. Data was presented as means ± SE (*P=0.0014, **P=0.0004; Student's t test, N= 5 or 6 per group). Two-way ANOVA analysis showed a significant effect of housing conditions ($F_{1,16}$=54.88, P<0.0001) and genotype ($F_{1,16}$=10.84, P<0.01), with no significant interaction among the two parameters ($F_{1,16}$=0.08642, P=0.7726), suggesting that the extent of increase in the enriched groups wass comparable. Scale bar =100 μm.

significant increase in cell proliferation after experience in an enriched environment is consistent across genotypes, and that APPswe/PS1ΔE9-expressing cells have the capacity to proliferate to the same extent as wild-type cells when given the appropriate stimuli.

2. **Newly-formed proliferative cells differentiate, survive and incorporate in the dentate gyrus of APPswe/PS1ΔE9 mice following EE.**

To determine whether the EE-induced increase in the number of proliferating cells in the SGL is manifested by an increase in the number of new neurons, brain sections from APPswe/PS1ΔE9 and nontransgenic mice were immunolabeled with antibodies raised against neural differentiation markers, doublecortin (DCX), an indicator for early neuronal differentiation and neuronal nuclei (NeuN), a neuronal marker for mature neurons. We showed previously that the number of new neurons, as detected by the number of newly born cell expressing BrdU and DCX (BrdU+/DCX+) was significantly decreased in the SGL of APPswe/PS1ΔE9 mice maintained in standard housing, compared with the wild-type mice housed in the same conditions (**Figure 4**). This suggests that APPswe/PS1ΔE9 mice exhibits impaired neuronal differentiation in the hippocampus. However, after the experience in an enriched environment, the number of new neurons increased significantly in both APPswe/PS1ΔE9 mice and their nontransgenic littermates (**Figure 8A, 8B**). Using two-way ANOVA analysis, the results showed that the extent of EE-induced increase in the number of new neurons relative to the number of new neurons in the corresponding control animals was comparable in both groups. This indicates that EE affects both genotypes to the same extent (no interaction between the two parameters, $F_{1,16}$=0.6603, *P*=0.4284) (**Appendix I, ANOVA Table 2**), and that experience in an enriched environment can rescue impaired formation of new neurons in the DG of APPswe/PS1ΔE9 mice.
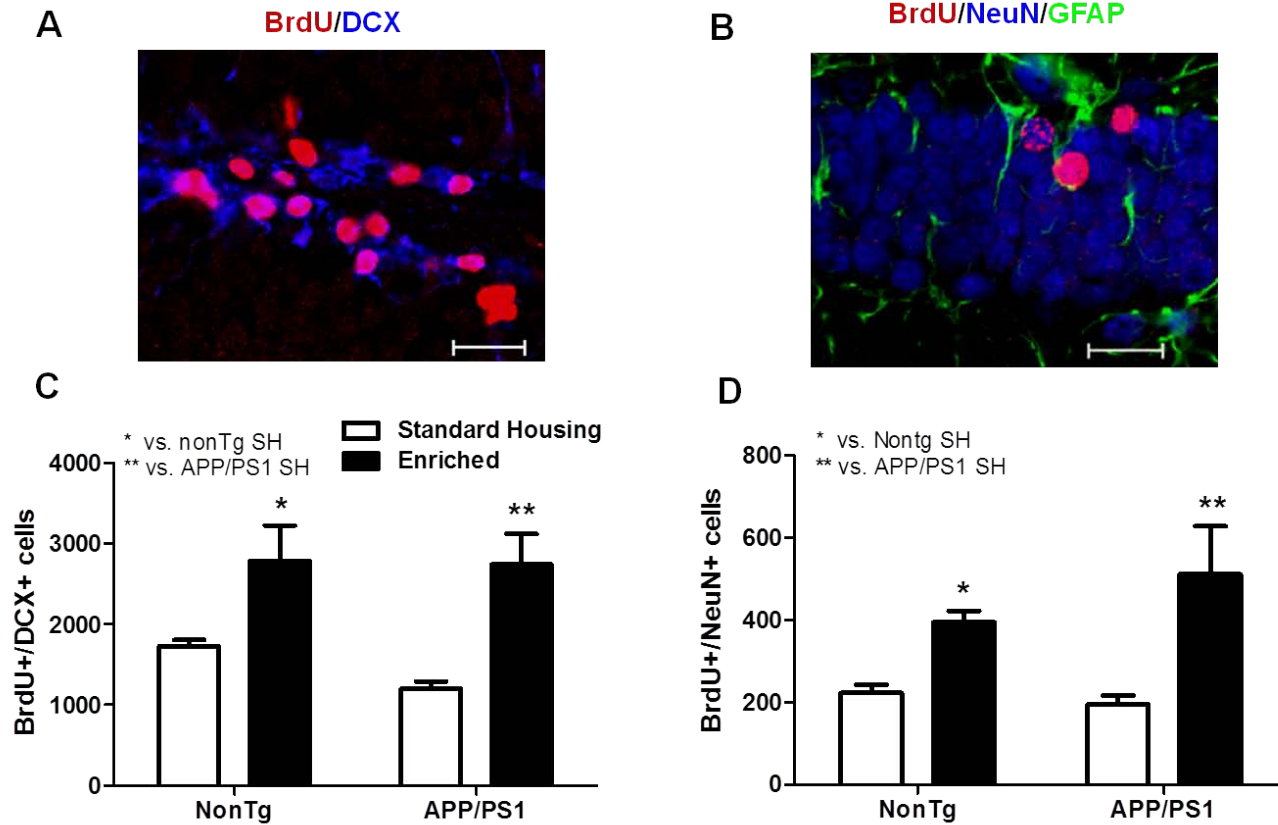
FIGURE 8 Increased neuronal differentiation and neuronal survival in the DG after experience in an enriched environment.

(**A**) High-power confocal images of cells co-expressing BrdU (red) and DCX (blue) in the SGL and (**B**) cells triple-labeled with BrdU (red), NeuN (blue) and GFAP (green) in the granular layer of DG. Scale bars= 20μm. (**C**) Number of newly formed neurons (BrdU+/DCX+) was significantly increased following EE (*P=0.0452, **P=0.0042, Student's t test, N=6 per group). (**D**) Experience in enriched environment promotes mature neurons (BrdU+/NeuN+) survival and migration into the granular layer of DG (*P=0.0022, **P=0.0382, Student's t test, N=6 per group). Two-way ANOVA showed no significant interaction between genotypes and housing conditions ($F_{1,16}$=0.6603, P=0.4284 for BrdU+/DCX+; $F_{1,12}$=0.1889, P=0.6716 for BrdU+/NeuN+), suggesting that the extent of increased in the enriched groups was comparable. All data was presented as mean ± SE.
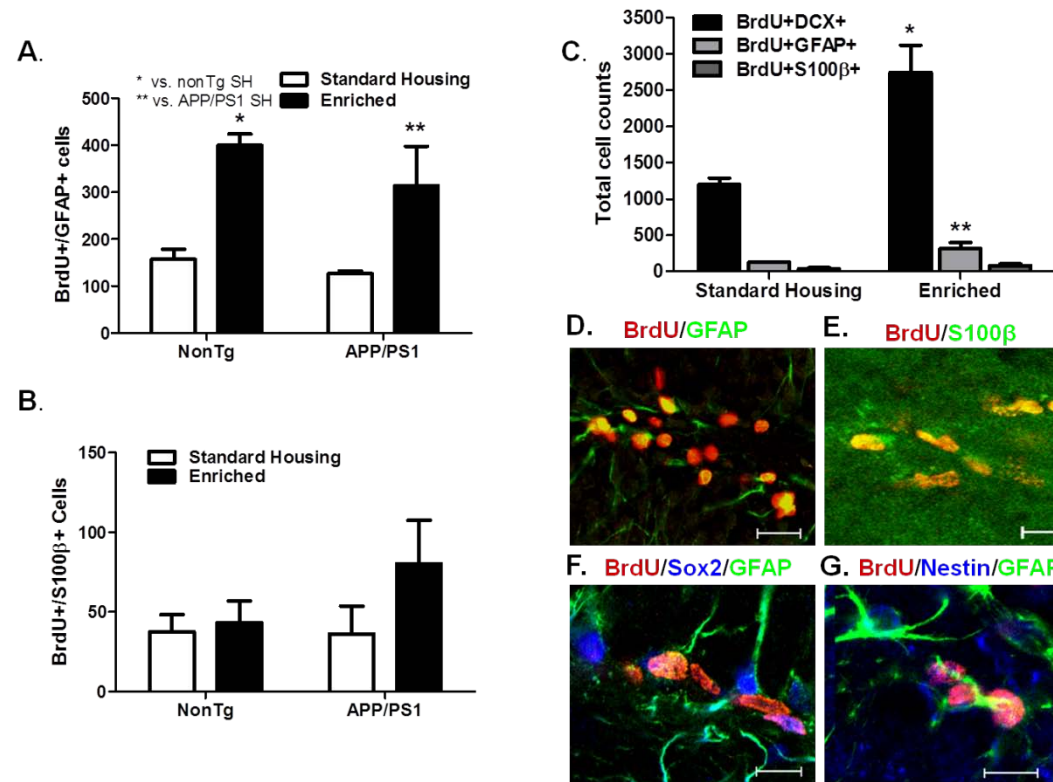
FIGURE 9 A selective increase in the number of glial cells in the brains of APPswe/PS1ΔE9 mice after experience in an enriched environment.

(**A**) Number of BrdU+/GFAP+ newly formed astrocytes in the DG was greatly increased in both NonTg and APPswe/PS1ΔE9 following EE, when compared with their standard housing controls (*P=0.0001,**P=0.0058; Student's t test, N=6 per group). (**B**) No significant increase in the number of BrdU+/S100β+ cells was observed following EE in the NonTg or the APPswe/PS1ΔE9 group (P=0.5945 and P=0.2575, respectively, N=6 per group), suggesting that EE induces signalings that upregulate specific subtypes of the glial population. All data were presented as means ± SE. (**C**) Comparison of the extent of increase in the number of lineage-specific BrdU+ cells examined in the DG of mice that experienced in an enriched environmental condition or standard housing. (**D, E**) Representative confocal image of cells co-expressing BrdU (red) and GFAP (green) (**D**) and BrdU(red) and S100β (green) (**E**) in the DG of APPswe/PS1ΔE9 mice. (**F, G**) Proliferating astrocytes in the SGL of APPswe/PS1ΔE9 co-localized with neural stem cell markers. BrdU+/GFAP+ cells co-immunolabeled with Sox2 (**F**) and Nestin (**G**). Scale bars = 20 μm (**D, E**); 10 μm (**F, G**).

To elucidate if the increase in the number of newly formed neurons is manifested by an increased number of newly formed mature neurons in the DG of APPswe/PS1ΔE9 mice, we analyzed the brain sections by immunohistochemistry one month after last BrdU injection for cells co-expressing BrdU and NeuN (BrdU+/NeuN+). The number of BrdU+/NeuN+ cells was significantly increased in the granular layer of DG of both nontransgenic and APPswe/PS1ΔE9 mice after experiencing in an enriched environment for 2 months. Similar to the previous finding, the extent of increase was comparable between the two genotypes ($F_{1,12}$=0.1889, P=0.6716) (**Figure 8B, 8D**). For detailed two-way ANOVA analysis, see **Appendix I, ANOVA Table 5**.

3. <u>Environmental enrichment increases the number of newly-formed astrocytic cells that co-express neural stem cell markers.</u>

To characterize the cell lineages of proliferating BrdU+ cells in the DG, brain sections were immunolabeled with Glial Fibrillary Acidic Protein (GFAP) marker and neural stem cell markers, such as SRY (sex determining region Y)-box 2 (Sox2) and Nestin. There was no significant difference in the number of newly formed astrocytes (BrdU+/GFAP+) between the nontransgenic and APPswe/PS1ΔE9 mice maintained in standard housing condition. However, experience in an enriched environment increased the number of newly formed astrocytes in both nontransgenic and APPswe/PS1ΔE9 mice (**Figure 9A**). To further characterize these BrdU+/GFAP+ cells, brain sections were triple-labeled with neural stem cell markers, Nestin and Sox 2. We showed that BrdU+/GFAP+ cells co-localized with nestin and Sox2, suggesting that these BrdU+/GFAP+ cells represent NPCs (**Figure 9F, 9G**). In contrast, examination of the number of newly formed S100β+ cells, a marker of mature astrocytes, revealed no significant difference in the number of BrdU+/ S100β+ cells

between different genotypes and housing conditions (**Figure 9B**), For detailed two-way ANOVA analysis, see **Appendix I, ANOVA Table 4**. This data suggested that EE only upregulates a selective target population of NPCs in the DG. The overall distribution of cells expressing different cell lineage markers is presented in **Figure 9C**.

In summary, these results suggest that the experience of APPswe/PS1∆E9 mice in an enriched environment rescued impairment in neurogenesis that were exhibited by the transgenic mice living in standard housing condition. Environmental enrichment induced an increase in neurogenesis in the APPswe/PS1∆E9 mice to an extent that was comparable to the one observed in the nontransgenic mice.

4. **Environmental enrichment attenuates amyloid pathology by reducing the extent of amyloid deposition and oligomeric Aβ levels in the cortex and hippocampus of APPswe/PS1∆E9 mice.**

To examine the effect of EE on amyloid pathology in APPswe/PS1∆E9 mice, brain sections of 3 month-old and 5 month-old APPswe/PS1∆E9 mice were immunolabeled with 3D6 antibody that recognized senile plaques. Brain sections of 3 month-old APPswe/PS1∆E9 mice didn't show 3D6 immunoreactivity (**Figure 10A**), this data is consistent with previous literature that the onset of amyloid deposition in APPswe/PS1∆E9 mice is around 4 months of age (Garcia-Alloza et al., 2006). At the age of 5 months, abundant amount of amyloid deposition was detected in both the cortex and hippocampus of APPswe/PS1∆E9 mice maintained in standard housing. Intriguingly, environmental enrichment effectively reduced the extent of amyloid deposition in cortex and hippocampus in our transgenic mice (Lazarov et al., 2005b).
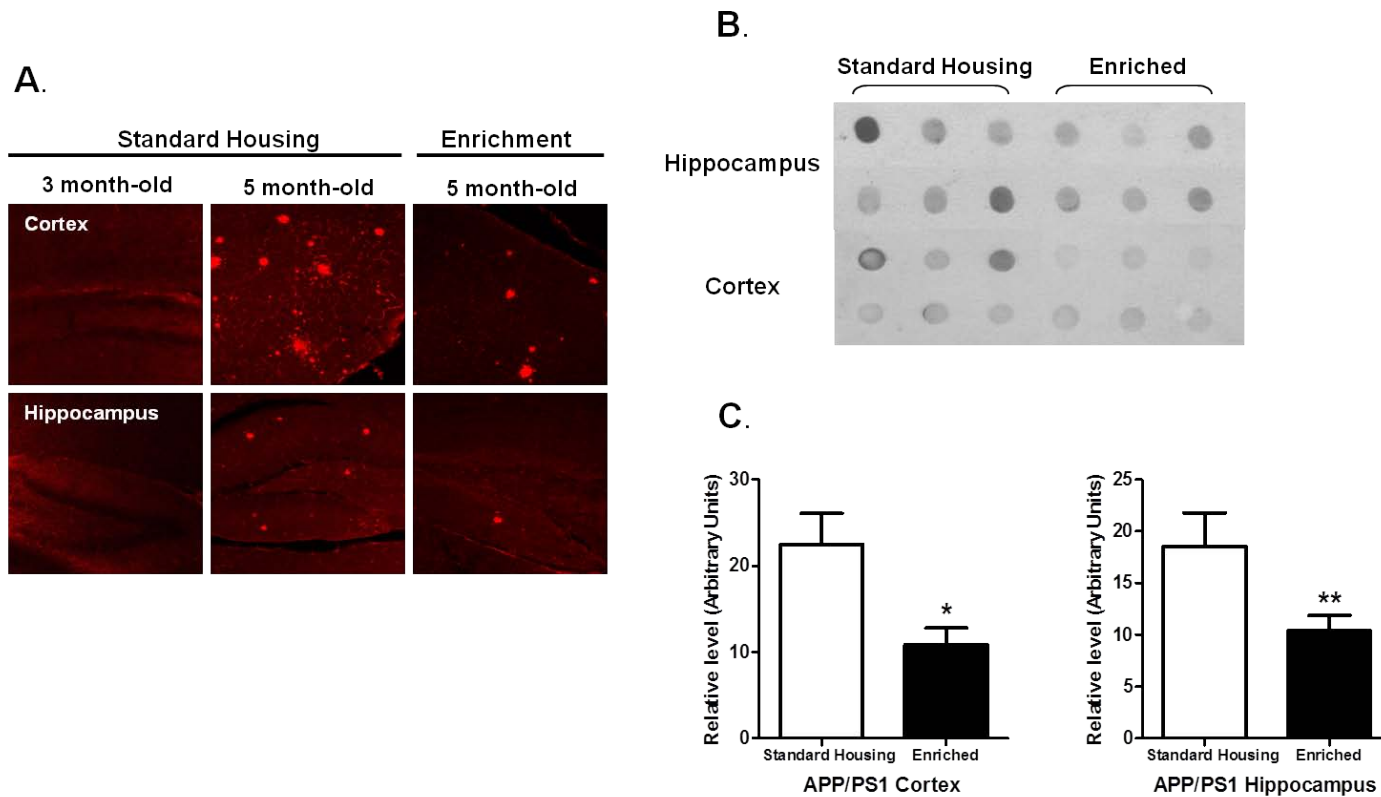
FIGURE 10 Environmental enrichment reduced the extent of amyloid deposition and levels of soluble oligomeric Aβ in the cortex and hippocampus of FAD-linked APPswe/PS1ΔE9 mice.

(**A**) Confocal images of brain section of 3 month-old and 5 month-old standard housing and enriched FAD-linked APPswe/PS1ΔE9 transgenic mice immunolabeled with anti-human Aβ 3D6 antibody. Mice were maintained in the standard housing condition. At three months of age, there was no sign of amyloid deposition in both the cortex and hippocampus of these mice. At the age of 5 months, both cortex and hippocampus exhibited abundant amount of amyloid plaques. Intriguingly, environmental enrichment significantly reduced the amount of amyloid deposition in both the cortex and hippocampus. (**B**) Representative dot blot analysis with conformation-specific antibody (A11) for the detection of amyloid-beta peptides of APPswe/PS1ΔE9 mice housed in a standard housing and in an enriched environment. (**C**) Quantifications of dot blot analysis showed significant reduction of oligomeric amyloid-beta levels in both cortex and hippocampus of APPswe/PS1ΔE9 mice following EE, as compared to standard housing condition (*$P<0.05$, **$P<0.05$, Student's t test, N=6 per group).

Soluble oligomeric forms of Aβ peptides, the precursors of amyloid deposits, are thought to be neurotoxic and are capable of impairing learning and memory (Cleary et al., 2005), synaptic plasticity (Li et al., 2009) and hippocampal LTP (Shankar et al., 2008). Because impaired neurogenesis and rescued neurogenic response were observed in APPswe/PS1ΔE9 mice maintained in standard housing and enriched environment at 2 months of age, which is 2-3 months before the onset of amyloid deposition, we examined whether EE can also attenuate the level of soluble oligomeric Aβ in our FAD-linked transgenic mice. Dot-blot analysis was performed using conformation-specific oligomeric antibody, A11, to detect the level of soluble oligomeric Aβ in the cortex and hippocamppus. As predicted, we observed a significant reduction in the levels of soluble oligomeric Aβ in both the cortex and hippocampus of APPswe/PS1ΔE9 mice experienced in an enriched environment (**Figure 10B**).

Taken together, these results suggest that experience in an enriched environment reduces the extent of amyloid deposition, as well as attenuates the levels of their neurotoxic precursors, oligomeric Aβ peptide, that can further aggregate to form senile plaques. These experiments further imply that after experience in an enriched environment, NPCs in APPswe/PS1ΔE9 mice are exposed to lower level of neurotoxic oligomeric Aβ.

5.   Environmental enrichment attenuates tau pathology by reducing hyperphosphorylated tau levels.

Tau hyperphosphorylation and the formation of neurofibrillary tangles are another important pathological hallmark of Alzheimer's disease. To examine whether the experience of APPswe/PS1ΔE9 mice in an enriched environment can attenuate tau pathology, we examined the level of tau phosphorylation by Western blot analysis. Using PHF-1 antibody that recognizes clinically-relevant tau phosphorylation at Ser-396 and Ser-404 epitopes, we

observed a significant reduction in levels of tau phosphorylation in both the cortex and hippocampus of APPswe/PS1ΔE9 mice following EE (**Figure 11A, 12A**). To examine whether EE-dependent reduction of tau phosphorylation is the result of a decrease in steady-state level of tau isoforms, we re-probed the blots with phosphorylation-independent Tau 5 antibody that recognizes the total tau levels. In both cortex and hippocampus, there was no significant change in the expression level of Tau 5 between the brain of standard housing and enriched mice, suggesting that the reduction in PHF-1 levels was due mainly to the effect of EE on tau hyperphosphorylation. We also observed no significant changes in the steady-state level of full-length APP across different housing conditions. Expression levels of actin were used as the loading controls for Western blot analysis. These findings indicate that environmental enrichment specifically and selectively modulates tau phosphorylation levels, possibly via the regulation of kinase activities.

6. <u>Environmental enrichment upregulates the expression of anterograde axonal transport motor protein, kinesin-1.</u>

Tau plays a major role in axonal transport (LaPointe et al., 2009). The increased levels of tau phosphorylation and oligomeric Aβ both have been shown to disrupt axonal transport in FAD models (Morfini et al., 2002a; Pigino et al., 2009). Because changes in axonal transport are also greatly associated with FAD pathology, and the expression of FAD-linked mutations can impair axonal transport (Lazarov et al., 2005a; Lazarov et al., 2007; Pigino et al., 2003), we examined whether expression levels of motor proteins critical for anterograde axonal transport were altered following experience in an enriched environment. Using H2 and 63-90 antibodies that specifically recognize kinesin-1 heavy chain (KHC) and kinesin-1 light chain (KLC), we observed a significant increase in the expression levels of both KHC and KLC in the cortex and hippocampus of APPswe/PS1ΔE9
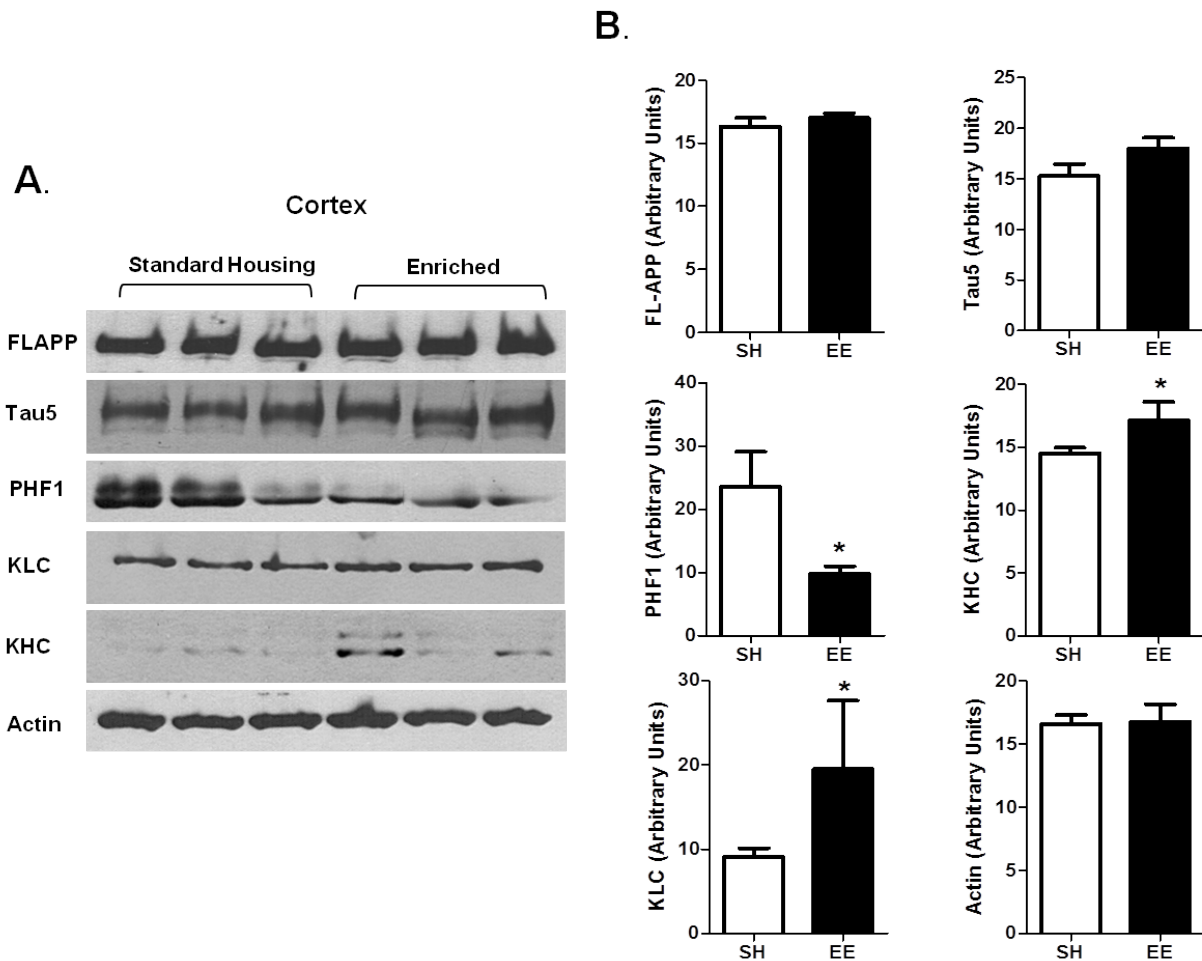
FIGURE 11 Environmental enrichment attenuates tau pathology by reducing hyperphosphorylated tau levels in the cortex.

(**A**) Representative Western blot analysis from the cortex of standard housing (SH) and enriched (EE) APPswe/PS1ΔE9 mice, showing the protein expression levels of full length APP (FL-APP) using anti-APP C-terminal 369 antibody; Tau 5, total tau protein; PHF-1, phosphorylated tau protein; KHC, kinesin-1 heavy chain using H2 antibody; KLC, kinesin-1 light chain using 63-90 antibody. (**B**) Densitometric quantification of protein expression levels in the cortex of standard housing and enriched mice, as detected by Western blot analysis. Data was presented as mean (arbitrary units) ± SE. *$P<0.05$, Student's t test, N=5 per group.
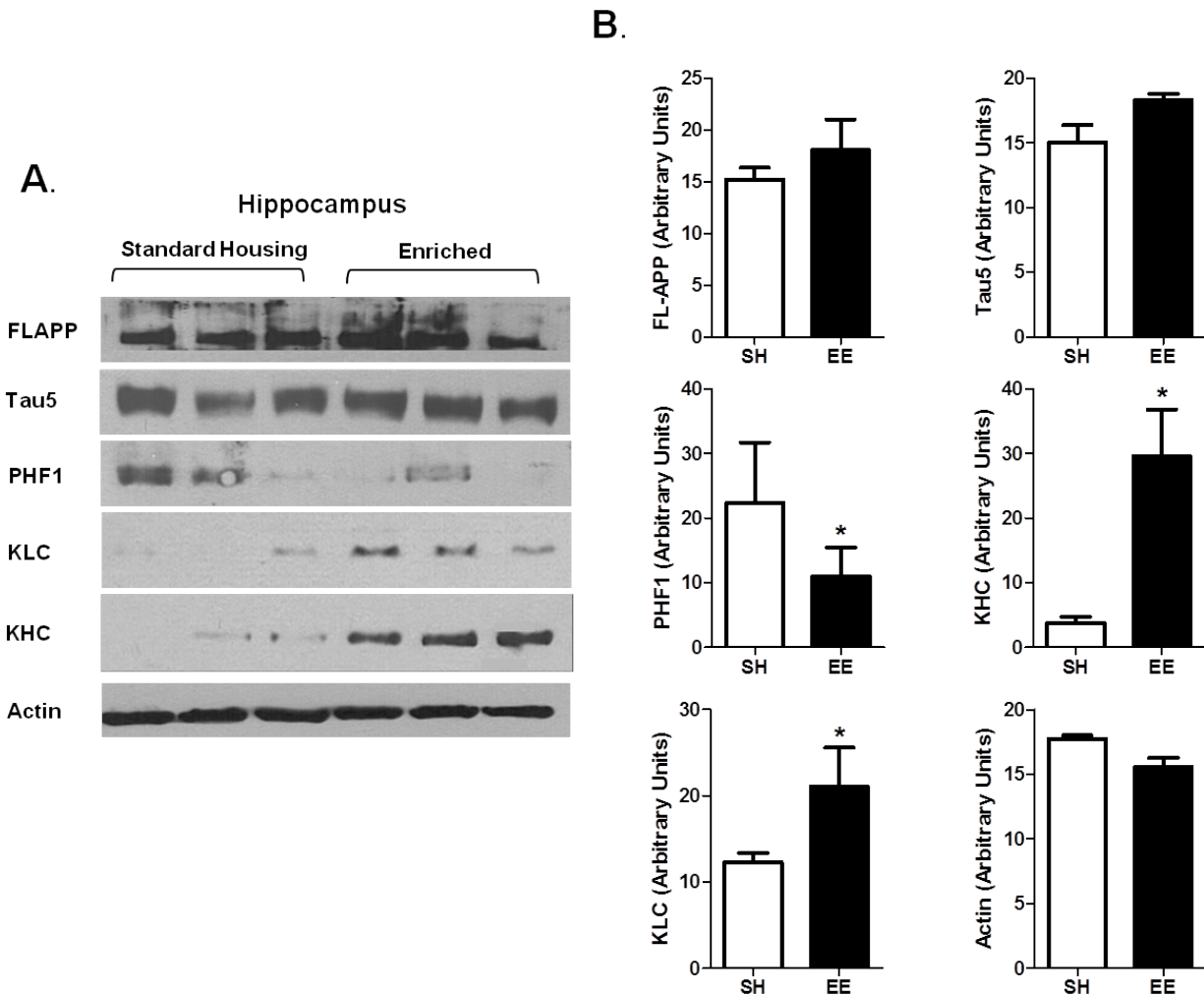
FIGURE 12 Environmental enrichment attenuates tau pathology by reducing hyperphosphorylated tau levels in the hippocampus.

(**A**) Representative Western blot analysis from the hippocampus of standard housing (SH) and enriched (EE) APPswe/PS1ΔE9 mice, showing the protein expression levels of full length APP (FL-APP) using anti-APP C-terminal 369 antibody; Tau 5, total tau protein; PHF-1, phosphorylated tau protein; KHC, kinesin-1 heavy chain using H2 antibody; KLC, kinesin-1 light chain using 63-90 antibody. (**B**) Densitometric quantification of protein expression levels in the cortex of standard housing and enriched mice, as detected by Western blot analysis. Data was presented as mean (arbitrary units) ± SE. *P<0.05, Student's t test, N=5 per group.

mice that experienced an enriched environment (**Figure 11A,12A**), This result suggests that anterograde axonal transport may be enhanced in the brains of these mice.

Taken together, these results suggest that experience of APPswe/PS1ΔE9 mice in a complex environment significantly attenuates tau hyperphosphorylation in the cortex and hippocampus without affecting steady-state levels of tau isoforms. Increased expression of kinesin-1 levels concomitantly with the attenuation of tau PHF-1 immunoreactivity implies that EE-induced alteration in kinase and phosphatase activity might be manifested by enhancement of axonal transport in the brains of APPswe/PS1ΔE9 mice.

7. <u>Environmental enrichment enhances hippocampal long-term potentiation (LTP).</u>

To determine whether EE stimulates synaptic plasticity in APPswe/PS1ΔE9 transgenic mice, electrophysiological recordings were made in the CA1 field of hippocampal slices prepared from APPswe/PS1ΔE9 mice after 1 month experience in an enriched environment or maintenance in a standard housing condition. Recordings were also made from nontransgenic littermate mice treated similarly. EE had no significant effect on input-output curves (**Figure 13A**) or paired-pulse facilitation (**Figure 13B, 13C**) in APPswe/PS1ΔE9 or nontransgenic mice. LTP was induced by TBS consisting of 2, 4, or 8 bursts. LTP after 2 bursts was small and unreliable and was not analyzed further. The results from 4- and 8-burst TBS are shown in **Figure 14**. A three-way analysis of variance was conducted to determine the contributions of number of bursts, genotype, and housing conditions on the degree of LTP measured 60 min post-TBS. No main effects of genotype ($F_{1,64}=0.12$) or burst number ($F_{1,64}=0.08$) were observed; however, there was a significant main effect of housing condition ($F_{1,64}=8.03$, *P*<0.01). No significant interactions were observed between any of the independent variables. These results indicate that experience
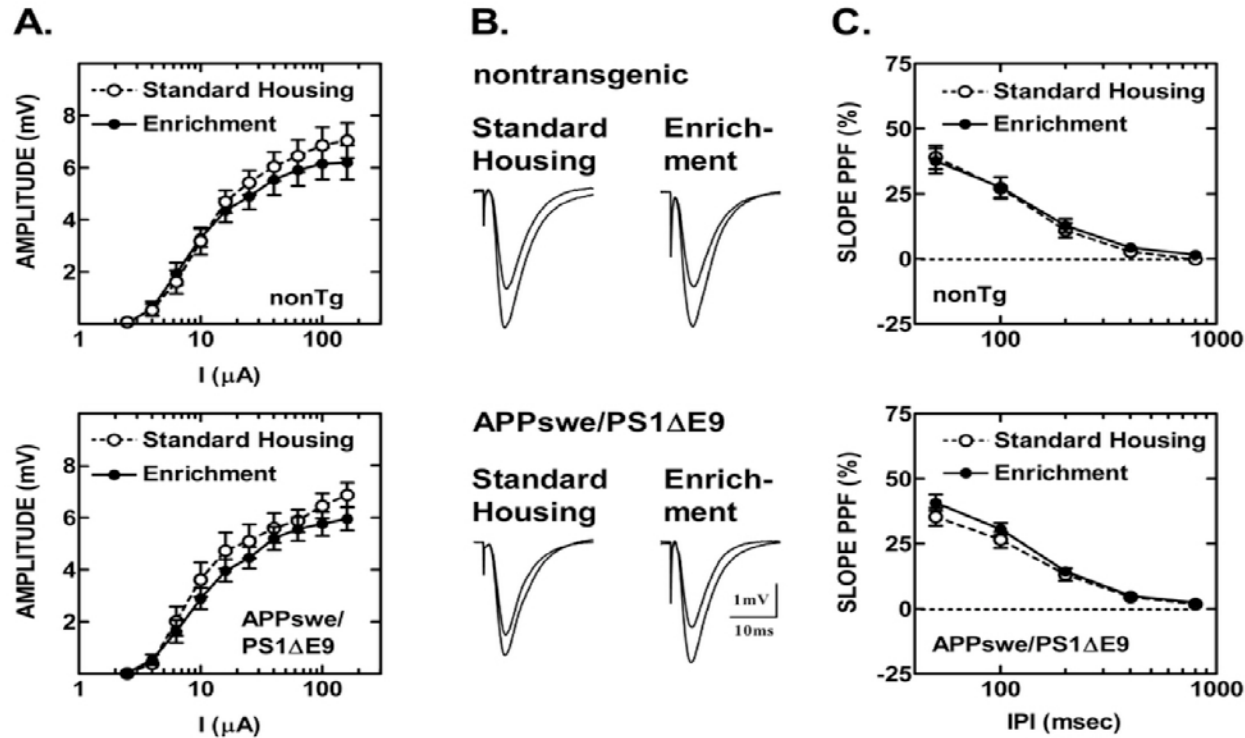
FIGURE 13 Synaptic transmission and short-term plasticity is normal in APPswe/PS1ΔE9 mice and unaffected by environmental enrichment.

(**A**) fEPSP input-output curves in the CA1 field of hippocampal slices from mice in standard housing conditions and after environmental enrichment. Schaffer/commisural synapses were stimulated with stimulus intensities of 2.5, 4.0, 6.3, 10, 16, 25, 40, 63, 100, and 160 mA. Graphs plot fEPSP amplitude (4 consecutive responses at each intensity in each slice). Values are means $\pm$ SE obtained from 1 slice from each Nontransgenic (top; N=9 for standard housing, N=9 for enrichment) and APPswe/PS1ΔE9 (bottom; N=10 for standard housing, N=15 for enrichment) mouse. ANOVA did not show any significant differences attributable to genotype ($F_{1,39}$=0.44, P>0.40) or housing condition ($F_{1,39}$=1.47, P>0.20); no significant interactions were observed between these factors and stimulus intensity. (**B**) Representative waveforms evoked by paired-pulse stimulation in mice with indicated genotype and housing conditions. (**C**) Facilitation curves generated by paired-pulse stimulation at indicated interpulse intervals (IPIs) in the same slices shown in **A**. No significant differences in facilitation were observed to be attributable to genotype ($F_{1,39}$=0.35, P>0.55) or housing conditions ($F_{1,39}$=0.70, P>0.40), and no interaction was observed between these factors and IPI.
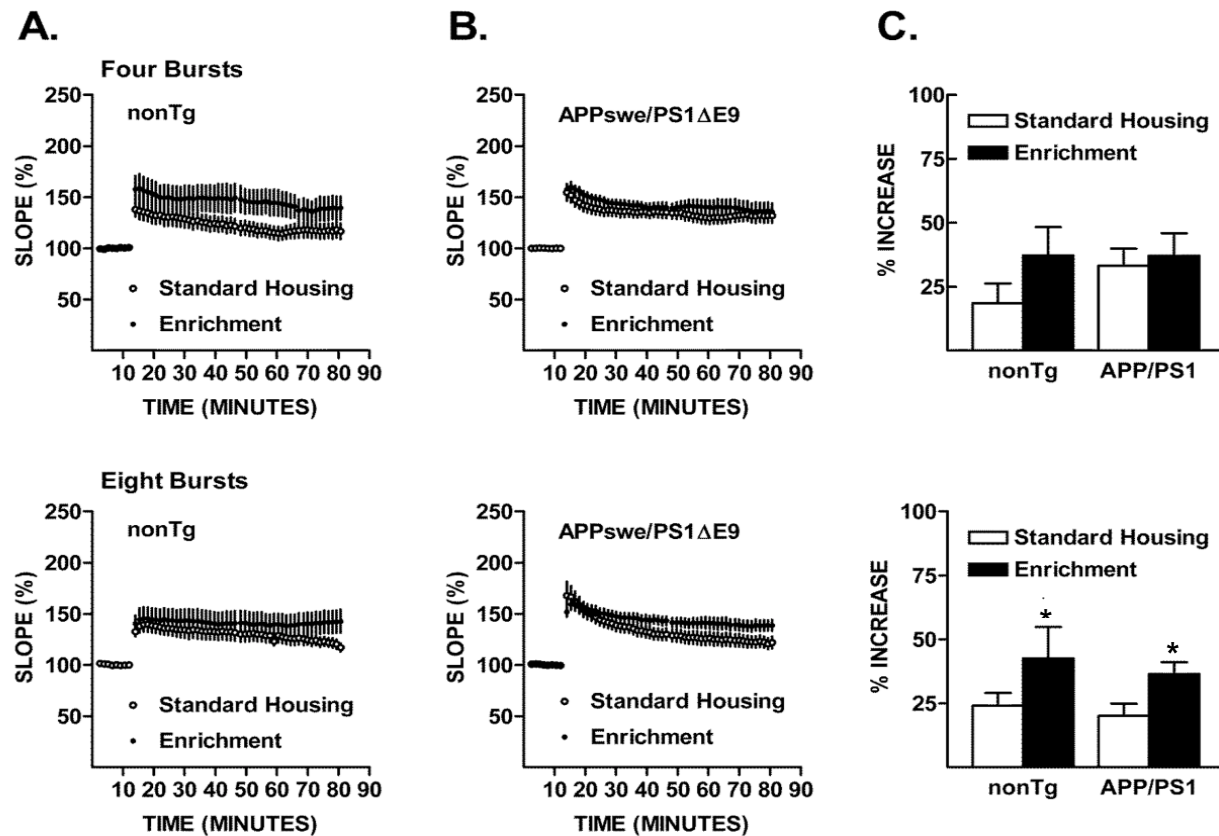
FIGURE 14 LTP is enhanced after environmental enrichment.

TBS consisted of 4 (top panels) or 8 (bottom panels) high-frequency bursts. (**A**) Comparison of LTP in nontransgenic mice in standard housing or after environmental enrichment. Graphs show fEPSP slope (mean ± SE) before and after TBS. (**B**) LTP in APPswe/PS1ΔE9 mice with standard housing or environmental enrichment. Graphs show fEPSP slope (means ± SE) before and after TBS. (**C**) Histograms show mean percentage of increase of potentiation at 60 min after TBS in each group (*$P<0.05$, Student's t test, Nontransgenic, N=9 for standard housing, N=9 for enrichment; APP/PS1, N=10 for standard housing, N=15 for enrichment).

of mice in an enriched environment enhanced LTP in both nontransgenic and APPswe/PS1ΔE9 transgenic mice (**Figure 14**).

C. Environmental enrichment upregulates BDNF levels and activates BDNF-dependent molecular signaling in nontransgenic and APPswe/PS1ΔE9 mice.

1. <u>**BDNF level is significantly increased in the hippocampus of nontransgenic and APPswe/PS1ΔE9 mice following experience in an enriched environment.**</u>

It has been shown repeatedly in many studies that exercise and environmental enrichment upregulate BDNF levels in the rodent brains (Adlard et al., 2005a; Oliff et al., 1998; Sartori et al., 2011). However, it is not known whether the level of BDNF can be modulated in our AD mouse model as young as 2 months of age. To address this, we determined the protein level of BDNF in the hippocampus by ELISA. In this experiment, we acquired six different groups of animal (i.e. singly-housed (SH) nontransgenic, singly-housed APPswe/PS1ΔE9, group-housed (GH) nontransgenic, group-housed APPswe/PS1ΔE9, enriched (EE) nontransgenic and enriched APPswe/PS1ΔE9). As expected, we observed a significant increase of BDNF level in hippocampus of nontransgenic and APPswe/PS1ΔE9 mice following experience in an enriched environment, compared to their littermates in singly- housed and group-housed standard housing condition (**Figure 15A**, *P<0.001). Comparison of singly-housed nontransgenic and APPswe/PS1ΔE9 mice did not reveal any significant change in BDNF level, suggesting no impairment in BDNF production in our FAD-linked mice at an early age.

Next, we examined the expression level of BDNF in the cortex of these six groups of mice. Interestingly, BDNF level in the cortex was not affected by experience in an enriched environment. We did not observe any significant changes in BDNF level in any of the six
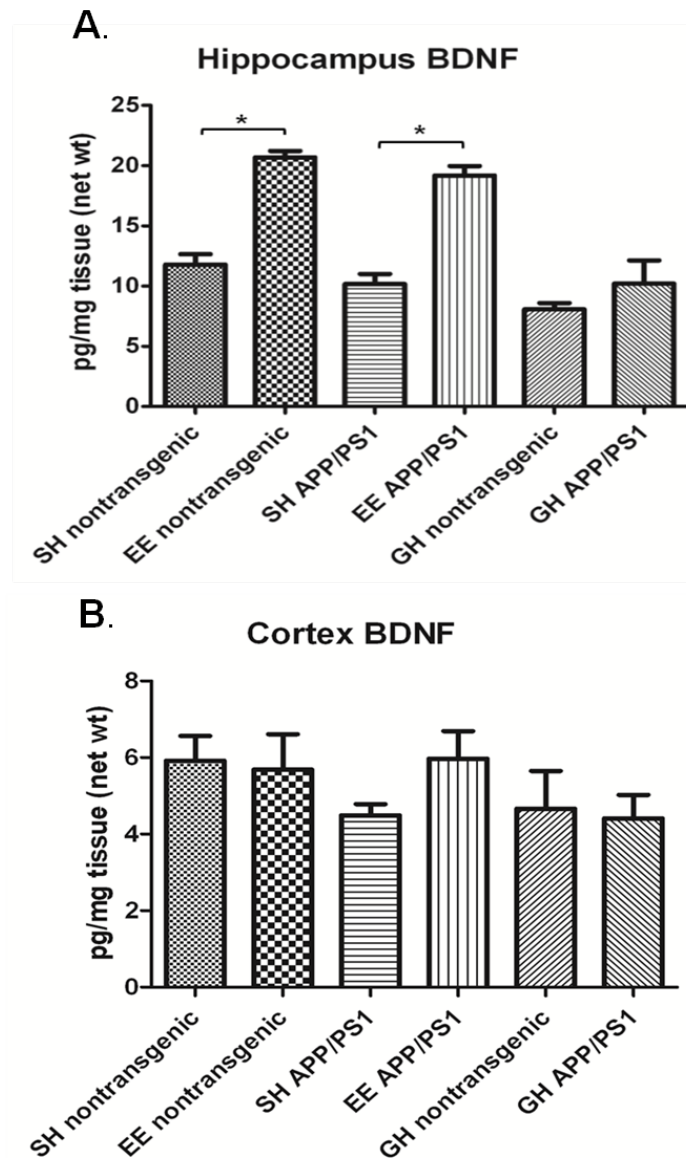
FIGURE 15 Alteration of BDNF protein levels in the hippocampus and cortex following environmental enrichment.

Nontransgenic and APPswe/PS1ΔE9 (APP/PS1) mice were housed in three different conditions; standard housing (SH), enriched environment (EE) and group-housing (GH). (**A**) The level of BDNF in the hippocampus was measured using ELISA. Nontransgenic and APPswe/PS1ΔE9 mice experienced in an enriched environment showed significant increased in BDNF levels, compared to their singly-housed controls (*$P<0.005$, one-way ANOVA, N=6 per group). To determine whether social interaction is an important component of EE, mice were group-housed in standard housing condition and the level of BDNF was measured. However, group-housing did not result in higher BDNF level, when compared to single-housed standard housing controls. (**B**) Alteration of BDNF levels is only specific to hippocampal and not the cortical regions of the enriched mice.

groups described earlier (**Figure 15B**). Detailed one-way ANOVA analyses are presented in **Appendix II**. This confirms our earlier studies that the effect of environmental enrichment is quite specific to brain regions. When it comes to BDNF levels, environmental enrichment mainly affected changes in the hippocampal and not the cortical regions in our FAD mice. In support of this, there is some evidence to suggest that the changes in the levels of neurotrophins in AD patients may be regional-specific (Hock et al., 2000; Narisawa-Saito et al., 1996).

2. <u>Gene expression level of neurotrophins is comparable in APPswe/PS1ΔE9 and nontransgenic littermates at 2 months of age.</u>

BDNF immunoreactivity is closely associated with senile plaques (Ferrer et al., 1999; Murer et al., 1999). Changes in BDNF expression have been reported in Alzheimer's disease patients (Murer et al., 2001). However, whether the levels of the neurotrophins and its receptors are increased (Angelucci et al., 2010; Burbach et al., 2004; Durany et al., 2000; Schulte-Herbrüggen et al., 2008), or decreased is still controversial in both human post-mortem Alzheimer's brains and in many AD mouse models. (Conner et al., 1997; Hock et al., 2000; Hock et al., 1998; Michalski and Fahnestock, 2003). In fact, depending on the regions studied, the levels of different neurotrophins in AD patients may vary (Hock et al., 2000; Narisawa-Saito et al., 1996).

To start to address this, we first asked whether neurotrophin levels and their signaling pathways are impaired early in life in APPswe/PS1ΔE9 mice. We started by comparing the gene expression levels of several neurotrophins and growth factor, namely BDNF, Neurotrophin-3 (NT3), nerve growth factor (NFG) and insulin growth factor-1 (IGF-1), in the hippocampus of nontransgenic and APPswe/PS1ΔE9 mice at 2 months of age, before
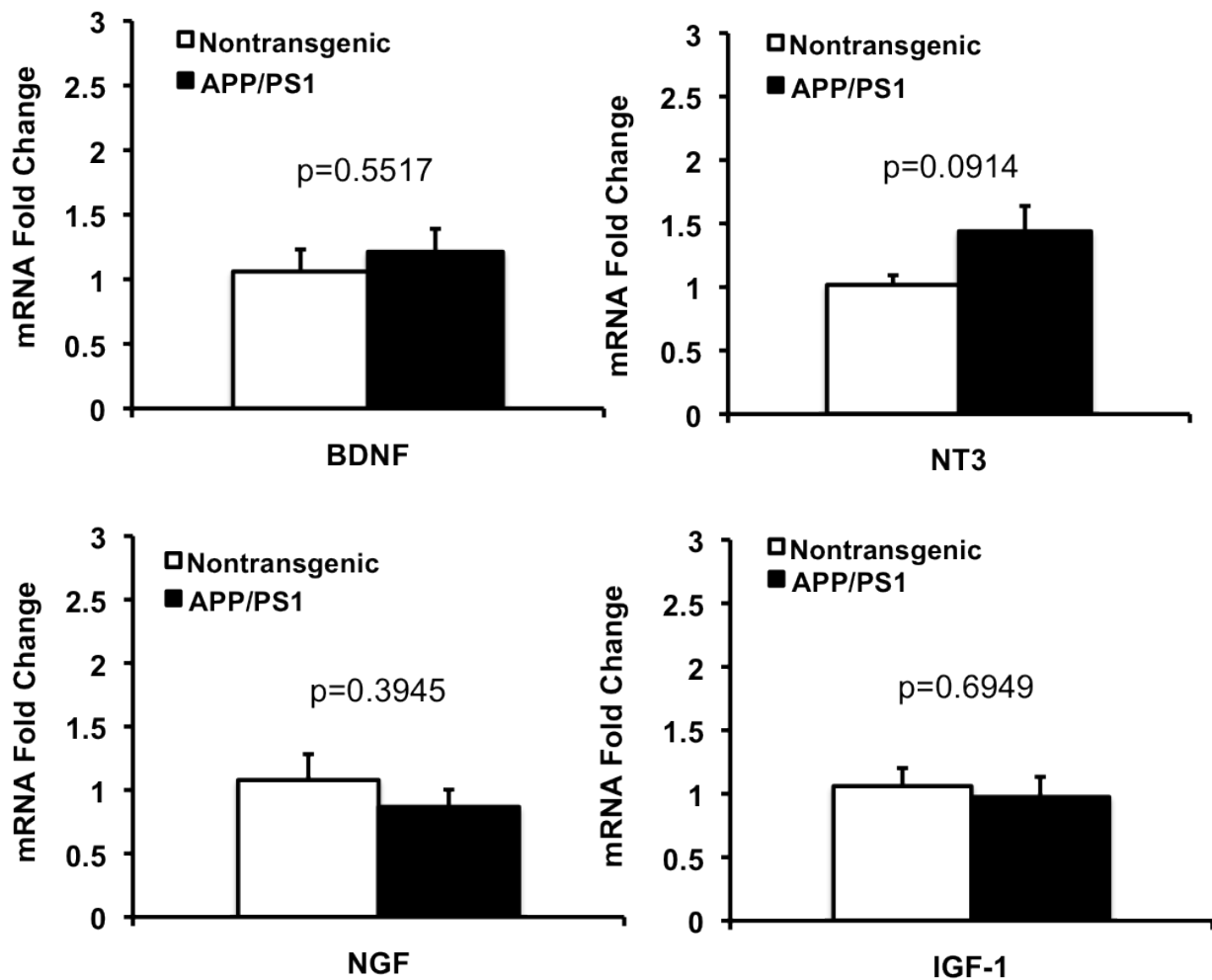
FIGURE 16 Gene expression of neurotrophins and growth factors is not altered at 2 months of age in APPswe/PS1ΔE9 mice compared to wild type.

mRNA levels of neurotrophins (BDNF, NGF and NT3) and growth factor (IGF-1) were measured by real time-RT-PCR in nontransgenic and APPswe/PS1ΔE9 mice housed in standard laboratory condition. mRNA expression level of BDNF, NGF, NT3 and IGF-1 was comparable between the two genotypes (BDNF, *P*=0.5517; NGF, *P*=0.3945; NT3, *P*=0.0914 and IGF-1, *P*=0.6949, Student's t test, N=6 per group).

the onset of AD pathology. While many studies have shown altered expression levels of neurotrophins in AD patients and AD mouse models at older ages, we did not observed any significant different in the gene expression level of BDNF, NT3, NGF and IGF-1 at early age by real-time RT-PCR (**Figure 16**).

3. Environmental enrichment upregulates BDNF and NGF gene expression in the hippocampus of nontransgenic and APPswe/PS1ΔE9 mice.

To further confirm the effect of EE on the expression level of BDNF and related neurotrophin families, we examined the mRNA expression levels of several neurotrophins and insulin-like growth factor 1 (IGF-1) in the hippocampus of nontransgenic and APPswe/PS1ΔE9 mice following EE by real time RT-PCR. As anticipated, we observed a two-fold increase in BDNF gene expression level in enriched mice compared to their standard housing littermates (**Figure 17A**). The extent of increase was comparable in the nontransgenic and APPswe/PS1ΔE9 mice, suggesting that upon induction by the appropriate stimuli, the cellular constituents of the hippocampus of FAD mice retain the ability to produce BDNF to the same extent as in wild-type mice. In addition, mRNA expression of NFG was also significantly upregulated by two folds in the enriched mice (**Figure 17B**).

Interestingly, we only observed a significant increase in NT-3 mRNA expression level in the nontransgenic mice that experienced an enriched environment, but not in enriched APPswe/PS1ΔE9 mice (**Figure 17C**). Conversely, IGF-1 mRNA expression was differentially upregulated only in the enriched APPswe/PS1ΔE9 mice, and not in the nontransgenic mice (**Figure 17D**). This may raise the possibility that EE exhibits differential effects on nontransgenic and APPswe/PS1ΔE9 mice. Nevertheless, further experiments are warranted in order to unravel the mechanisms underling these differences.
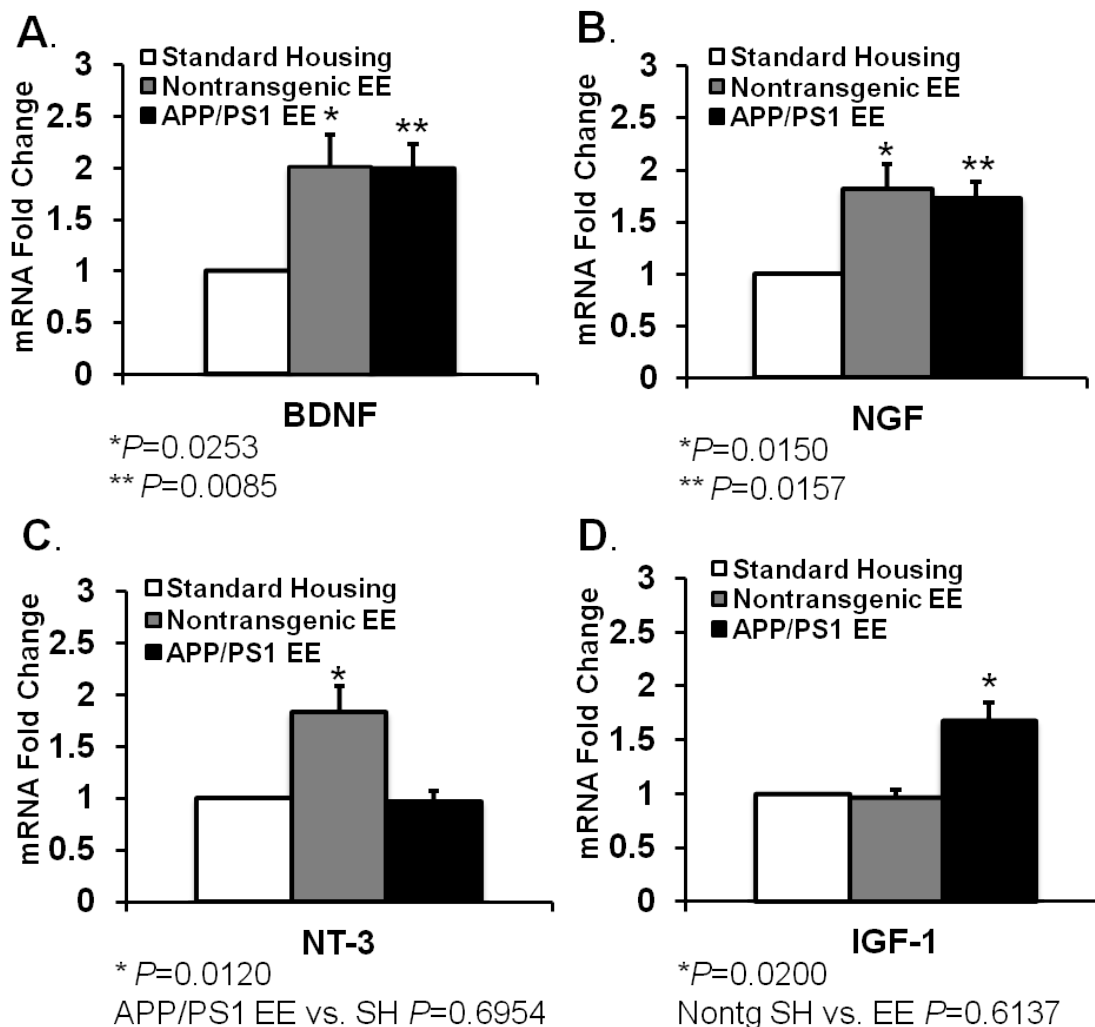
FIGURE 17 mRNA expression levels of neurotrophins and IGF-1 in the hippocampus following environmental enrichment.

mRNA levels of neurotrophins (BDNF, NGF and NT3) and insulin-like growth factor 1 (IGF-1) were measured by real time-RT-PCR in nontransgenic and APPswe/PS1ΔE9 mice following experience in an enriched environment. Standard housing groups were used as controls (no change in mRNA level) against the effect of environmental enrichment. (**A, B**) Environmental enrichment upregulated BDNF and NGF mRNA expression levels in APPswe/PS1ΔE9 and their nontransgenic littermates. (**C**) NT-3 mRNA expression level was significantly increased only in the enriched nontransgenic group. (**D**) Interestingly, IGF-1 mRNA expression level was induced by environmental enrichment in APPswe/PS1ΔE9 mice. (N=6 per group, Student's t test, results were significant when $P > 0.05$ in all groups).

4. **<u>Environmental enrichment modulates gene expression of molecular targets</u>**

    <u>associated with BDNF signaling pathways.</u>

To gain more insight into the EE-modulated downstream effectors of BDNF, we studied gene expressions of BDNF-associated targets by quantitative real-time RT-PCR. First, we examined the gene expression of BDNF receptor, TrkB. We showed that TrkB mRNA level was significantly upregulated following environmental enrichment (**Figure 18A**). Since EE was able to upregulate BDNF level and induced neuroplasticity in our AD mice, the increase in TrkB mRNA expression level was expected. The expression of cAMP response element-binding (CREB) is critical for learning and for the formation of long-term memory (Sakamoto et al., 2011). BDNF-mediated TrkB activation has been shown to promote neuronal synaptic activity through activation of the transcription factor, CREB, which drives the expression and activation of intracellular signaling pathways via the actions of two types of glutamate-gated ion channels, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. To test the hypothesis that EE promotes neuroplasticity via upregulation of BDNF-dependent signaling pathways, we examined the gene expression of CREB following EE. We showed that EE upregulated mRNA expression level of CREB by 3.5 folds (**Figure 18B**), possibly through the activation of BDNF via TrkB receptor. However, we did not observe any significant changes in the mRNA expression levels of CREB-binding protein (CBP) or protein kinase C (PKC) in the enriched mice (**Figure 18C, 18D)**.
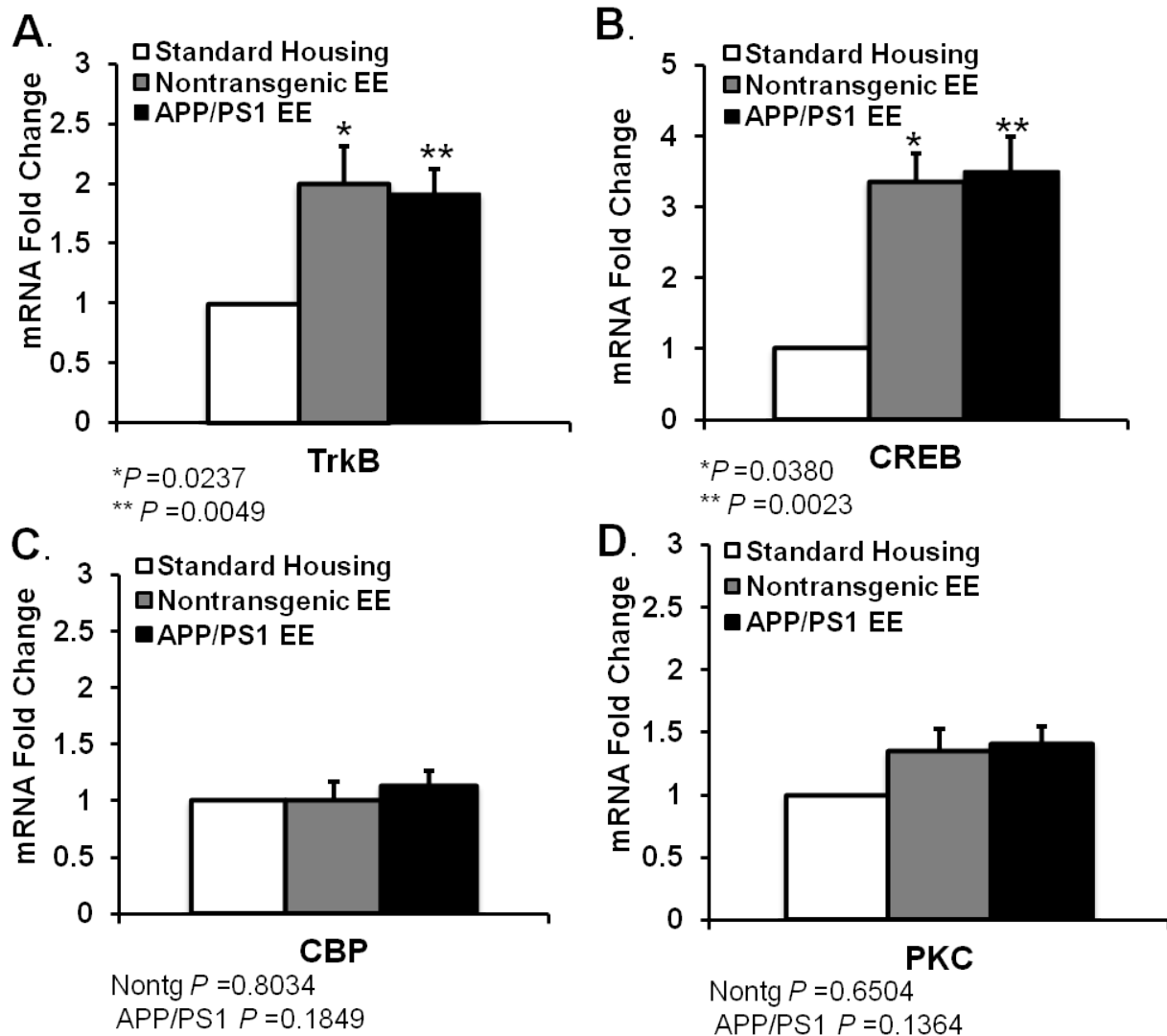
FIGURE 18 Expression level of molecular targets associated with BDNF-TrkB pathway are upregulated following environmental enrichment.

mRNA levels of BDNF-associated molecular targets (TrkB, CREB, CBP and PKC) were measured by real time-RT-PCR in the nontransgenic and APPswe/PS1ΔE9 mice following environmental enrichment. Standard housing groups were used as controls. (**A**) Environmental enrichment upregulated TrkB receptor mRNA levels in the hippocampus of APPswe/PS1ΔE9 and their nontransgenic littermates. It also upregulated the expression level of its downstream transcription factor, CREB (**B**). (**C,D**) No significant difference in CBP and PKC mRNA expression levels were detected following EE. (Student's t test, results were significant when $P>0.05$ in all groups).

Previously, we have shown that EE upregulated hippocampal LTP (**Figure 14**), suggesting a role for EE in regulating synaptic plasticity. BDNF increases the members of the AMPA receptor family, GluR1 and GluR2 mRNA and their subunits levels in the hippocampal neurons, and it also increases the mRNA and protein levels of ionotropic glutamate receptor, NMDA receptor subunits, NR1, NR2A and NR2B (Caldeira et al., 2007a; Caldeira et al., 2007b). By increasing the number of NMDA receptors, BNDF thereby upregulates receptor activity and promotes LTP formation. To examine whether EE can induce the expression of NMDA and AMPA receptors in APPswe/PS1ΔE9 mice, we examined the gene expression of NMDA1 and GluR1 receptors following EE. We showed that mRNA expression of NMDA receptor (**Figure 19A**), but not AMPA receptor GluR1 (**Figure 19B**), was significantly induced following EE.

Calcium-calmodulin-dependent protein kinase IV (CaMKIV) subunit has emerged as the most important Ca2+-activated CREB kinase *in vivo* (Ho et al., 2000; Kang et al., 2001). Therefore we also sought to examine the expression of CaMKIV in the hippocampus of mice following EE. CaMKIV acts as a kinase for the CREB binding protein (CBP). CBP is known as a transcriptional co-activator that interacts with CREB and proteins in the basal transcriptional complex. Interestingly, CBP is required for EE-induced neurogenesis and cognitive enhancement (Lopez-Atalaya et al., 2011). However, we didn't observe any significant increase in the mRNA level of CaMKIV or CBP following EE (**Figure 19E, 18C**).

Another important mediator of learning and memory is the calcium-calmodulin-dependent protein kinase II (CaMKII) subunit. It has been shown that CaMKII regulates the ion channel properties and synaptic trafficking of AMPA receptors during hippocampal LTP (Barria et al., 1997; Derkach et al., 1999; Esteban, 2003). However, gene expression level detected by real time RT-PCR did not show any significant changes in CaMKII mRNA level

(**Figure 19F**). Although postsynaptic density protein 95 (PSD-95) is a well-characterized postsynaptic marker for plasticity, neither did we detect any changes in the mRNA expression level of PSD-95 in animals that experienced EE, compared to their standard housing controls.

It is not known whether EE-induced BDNF increase is due to the increased production of BDNF or the increased processing of immature proBDNF into mature BDNF. Interestingly, studies have suggested that physical exercise affects hippocampal plasticity by promoting proBDNF proteolytic cleavages, thereby increasing the level of mature BDNF production (Ding et al., 2011; Sartori et al., 2011). This led us to examine the expression level of tissue-type plasminogen activator (tPA), a molecule that is responsible for the proteolytic cleavage of proBDNF. Interestingly, we observed a significant upregulation of tPA mRNA expression level in the "enriched" mice, suggesting that the proteolytic processing of proBDNF to mature BDNF may be enhanced following EE (**Figure 19C**).

D. Environmental enrichment downregulates kinase activities involved in tau phosphorylation.

1. <u>Activity of several kinases associated with AD pathogenesis is increased with aging.</u>

Numerous studies in FAD mouse models suggest that the regulation of GSK3β activity plays a critical role in the pathogenesis of Alzheimer's disease (Hernandez et al., 2009; Hernandez et al., 2010; Kaytor and Orr, 2002; Ryder et al., 2004). Addressing the level of GSK3β activity in post-mortem brain tissue is technically challenging. Nevertheless, indirect evidence did suggest that GSK3β activity might be increased in AD (Blalock et al., 2004; DaRocha-Souto et al., 2011; Hye et al., 2005; Leroy et al., 2007; Pei et al., 1997). Whether or not there is an age-associated increase of GSK3β activity in AD brains remains
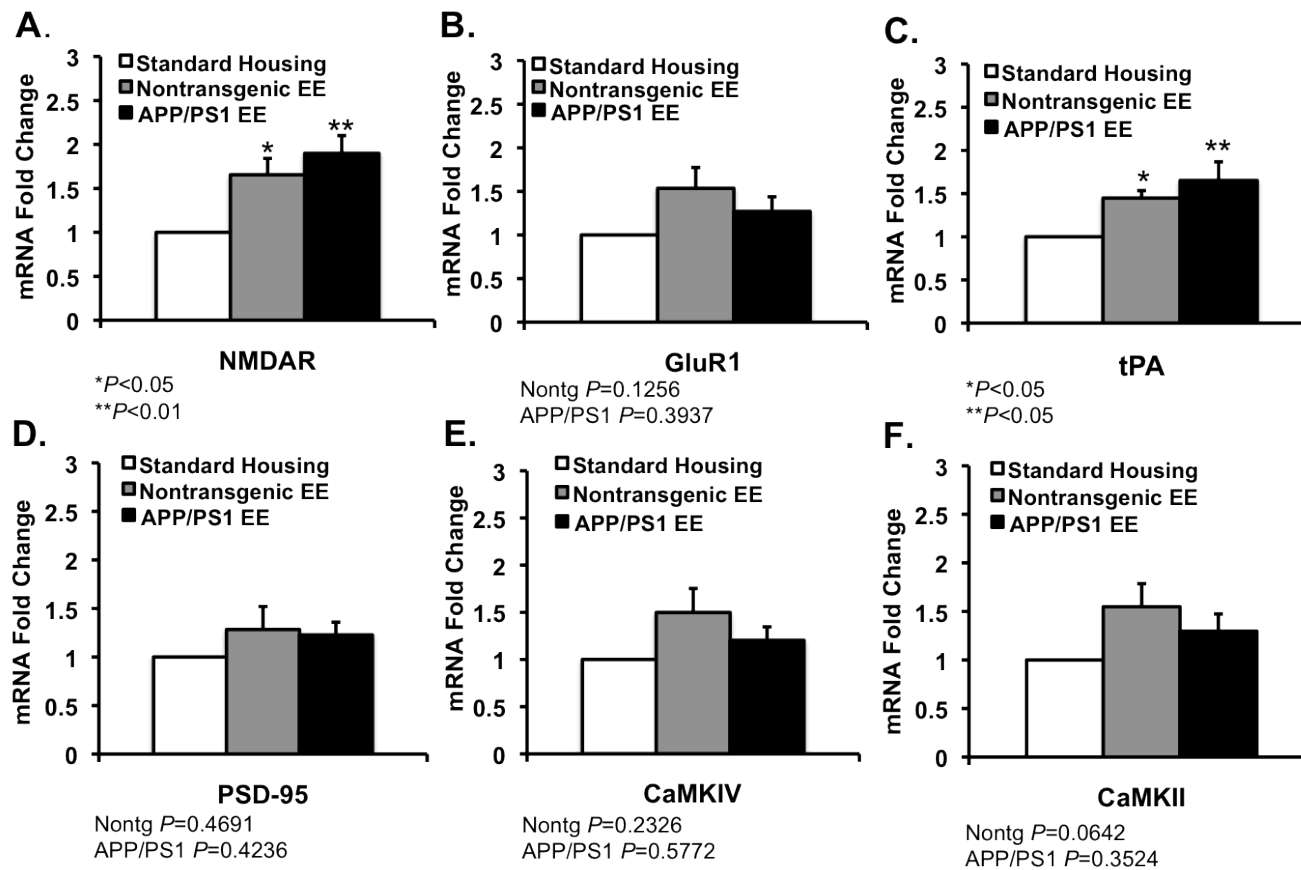
FIGURE 19 Gene expression of molecular targets associated with synaptic plasticity and proBDNF processing.

mRNA levels of BDNF-associated molecular targets (NMDAR, GluR1, PSD-95, CaMKIV, CaMKII and tPA) were measured by real time-RT-PCR in the nontransgenic and APPswe/PS1ΔE9 mice following EE. Standard housing groups were used as controls (with no change in mRNA level) against the effects of environmental enrichment. (**A**) Environmental enrichment upregulated NMDA receptor levels in APPswe/PS1ΔE9 and their nontransgenic littermates, however it did not change the expression levels of GluR1 (**B**). (**D,E,F**) Quantitative real-time RT-PCR also did not show any significant differences in PSD-95, CaMKIV and CaMKII mRNA expression levels following EE. (**C**) Interestingly, environmental enrichment upregulated the expression of tPA, indicating an increased proBDNF cleavage following EE. All data was analyzed by Student's t test. Results were significant when $P > 0.05$ in all groups.

inconclusive. In addition, it is not clear if the activity of other kinases associated with tau phosphorylation can be changed as a function of age and/or disease progression. To address this question, we examined the protein expression level of several tau-associated kinases at 2 and 6 months of age, pre- and post-onset of amyloid deposition, respectively. Hippocampal protein extracts of APPswe/PS1ΔE9 mice were analyzed by Western blot analysis for the expression levels of pGSK3β, pAkt and CK2. The expression of pGSK3β serine 9 represents the inactive form of GSK3β kinase (Frame et al., 2001). Likewise higher the expression level of pGSK3β serine 9 indicates reduced GSK3β activity. The result suggested an increase in GSK3β activity in APPswe/PS1ΔE9 mice at 6 months of age compared to 2 months of age, as shown by the decreased of pGSK3β immunoreactivity on Western blot analysis (**Figure 20A,B**).

One of the major upstream modulator of GSK3β in the BDNF-dependent signaling pathway is Akt, also known as protein kinase B. Akt can directly phosphorylate GSK3β and inactivates its activity. Like GSK3β, the activity of Akt is also regulated by phosphorylation by its upstream regulator, phosphoinositol-3 kinase (PI3K). However, unlike GSK3β, phosphorylation of Akt by PI3K increases its kinase activity. For this reason, we also examined the expression of Akt in the cortex and hippocampus of these mice. We observed a significant decrease of pAkt expression level in the 6 months old mice compared to 2 months old mice, supporting the evidence that Akt directly modulates GSK3β phosphorylation in the BDNF-dependent signaling pathway (**Figure 20C**). Casein kinase 2 (CK2) is another important serine/threonine kinase implicated in AD (Hanger et al., 2007; Walter et al., 2000). Interestingly, we showed that the kinase expression level of CK2 was decreased with aging in APPswe/PS1ΔE9 mice (**Figure 20D**). It is worth to mention that
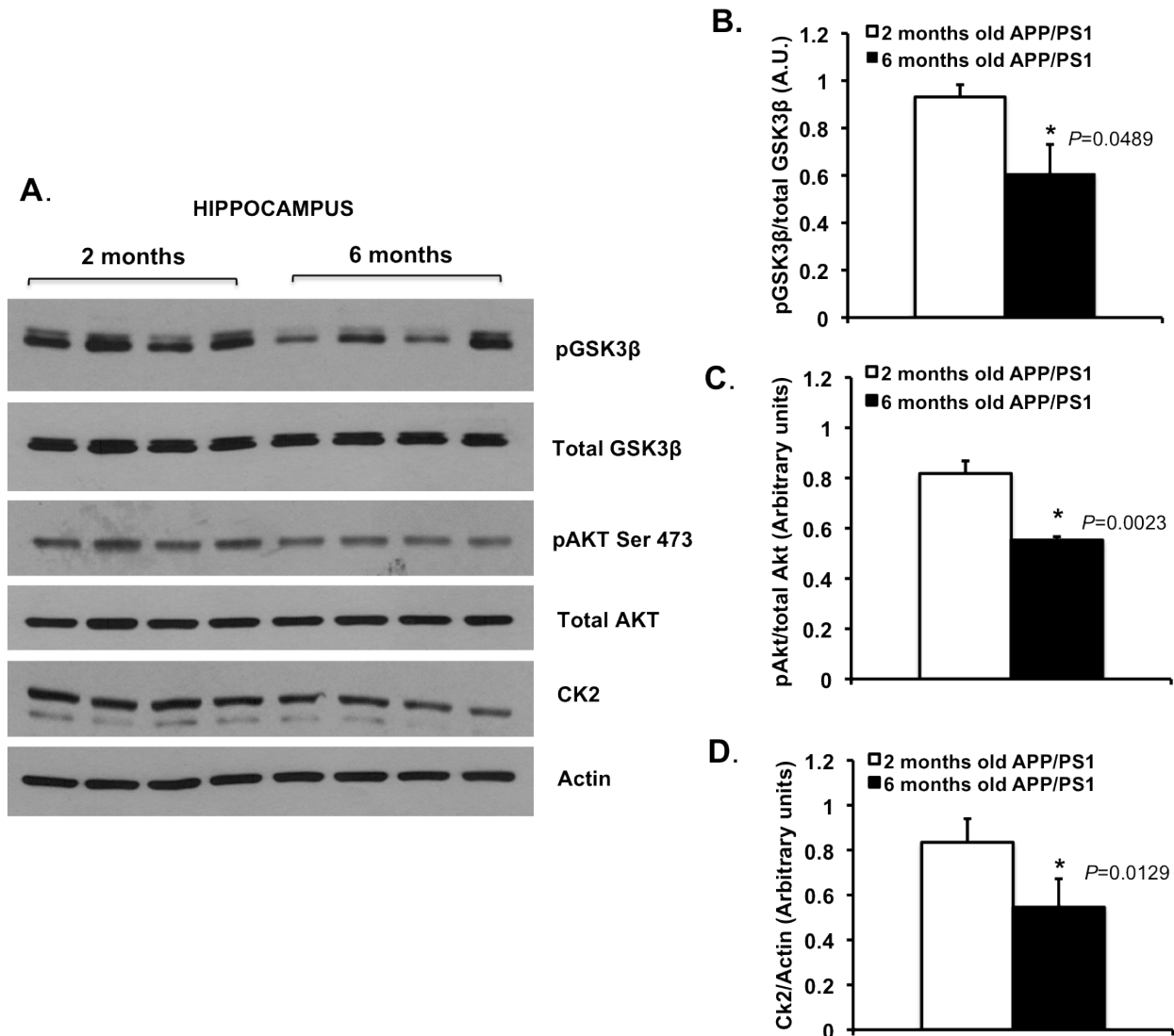
FIGURE 20 Expression level of several kinases associated with AD pathology is altered during aging.

(**A**) Representative immunoblots of hippocampal extracts from 2 month-old and 6 month-old APPswe/PS1ΔE9 mice for GSK3β, Akt and CK2 expression levels. The expression of phospho-GSK3β Ser-9 represents the inactive form of GSK3β kinase, such that higher expression level of pGSK3β serine 9 indicates a reduced GSK3β activity. On the contrary, the expression of pAkt Ser-437 represents the active form of Akt kinase, such that higher expression level of pAkt indicates an increased activation of Akt. The expression level of CK2 directly reflects its activity level. (**B, C, D**) Densitometric quantification of the Western blot was performed using Image J software, where pGSK3β was normalized to total GSK3β level, pAkt was normalized to total Akt level and CK2 was normalized to actin level. Data was presented as mean (arbitrary units) ± SE. *$P>0.05$, Student's t test, N=4 per group.

GSK3β

A.   **Cortex**

B.   **Hippocampus**

C.

D.

FIGURE 21 Expression level of GSK3β is comparable in nontransgenic and APPswe/PS1ΔE9 mice at 2 months of age.

Representative immunoblots of brain extracts from cortex (**A**) and hippocampus (**B**) of the nontransgenic and APPswe/PS1ΔE9 mice for GSK3β expression level. (**C, D**) Densitometric quantification of the Western blot was performed using Image J software, where pGSK3β serine 9 expression level was normalized to total GSK3β expression level. Data was presented as mean (arbitrary units) ± SE. P-values were as indicated on the graph, Student's t test, N=4 per group.

Akt
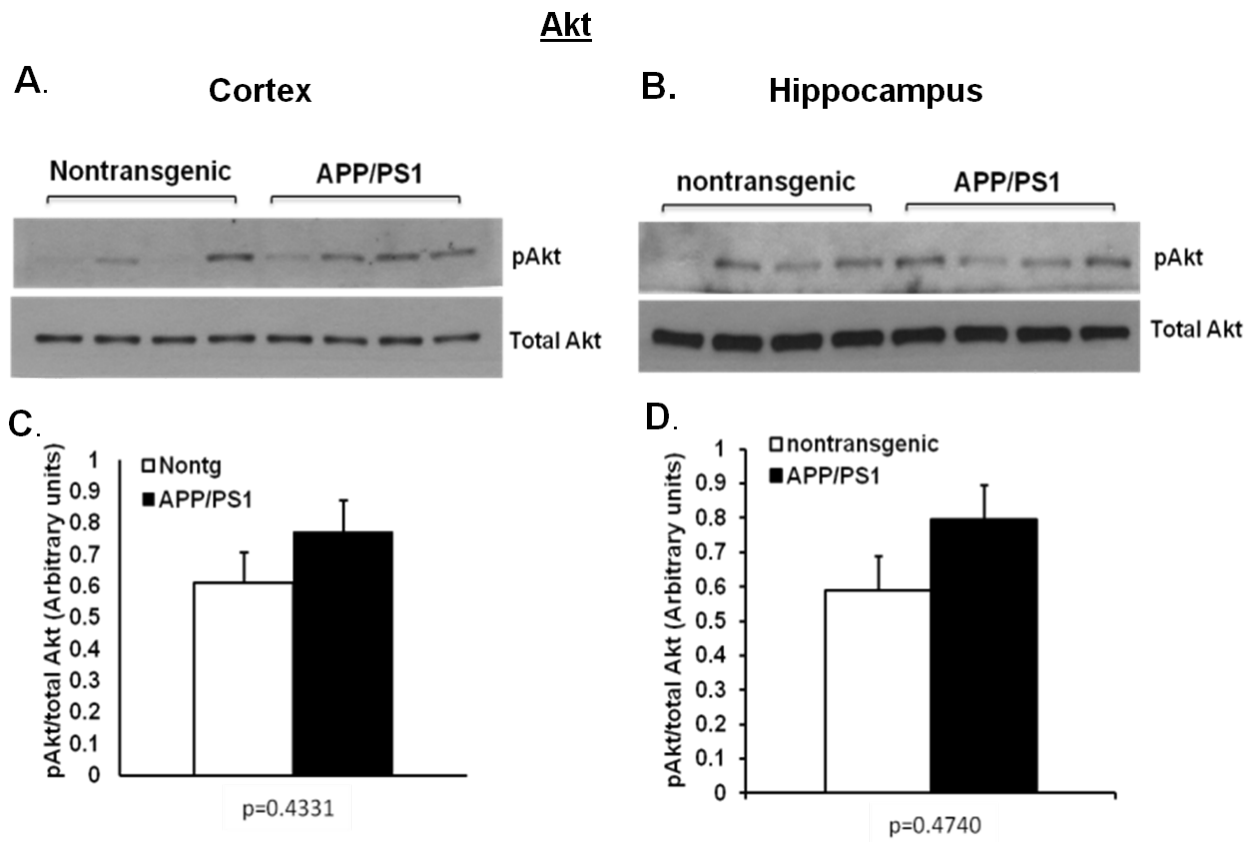
FIGURE 22 Expression level of Akt is comparable in nontransgenic and APPswe/PS1ΔE9 mice at 2 months of age.

Representative immunoblots of protein extracts from cortex (**A**) and hippocampus (**B**) of the nontransgenic and APPswe/PS1ΔE9 mice for Akt expression level. (**C, D**) Densitometric quantification of the Western blot was performed using Image J software, where pAkt serine 437 expression level was normalized to total Akt expression level. Data was presented as mean (arbitrary units) ± SE. P-values were as indicated on the graph, Student's t test, N=4 per group.

antibody (CKIIα) that we used to analyze CK2 cannot be used as a direct indicator for kinase activity level.

2. **Expression level of GSK3β and Akt are comparable in APPswe/PS1ΔE9 and nontransgenic mice at 2 months of age.**

To determine whether PI3K/Akt and GSK3β signaling pathways are compromised in FAD mice, we compared their expression levels in APPswe/PS1ΔE9 and wild type littermates maintained in the standard housing condition by Western blot analysis (N= 4 or 5 per group). The immunoblots showed no significant difference in the levels of GSK3β in the cortex and hippocampus in 2 months old mice (**Figure 21**). There was also no significant difference in the expression level of Akt in the cortex and hippocampus of these mice (**Figure 22**). This suggests that these signals are not compromised in the young APPswe/PS1ΔE9 mice.

3. **Environmental enrichment downregulates GSK3β activity in the nontransgenic, but not in the APPswe/PS1ΔE9 mice.**

To examine whether EE regulates the level of tau-associated kinases, we examined the protein expression levels of GSK3β in the cortex and hippocampus of the nontransgenic and APPswe/PS1ΔE9 mice following experience in an enriched environment (N= 4 or 5 per group) by Western blot analysis. We showed that the expression levels of pGSK3β was significantly upregulated in both the cortex and hippocampus of nontransgenic mice following EE (**Figure 23A, 24A**), strongly suggested a significant reduction of GSK3β activities following EE. However, this effect was not observed in FAD-linked APPswe/PS1ΔE9 mice following EE. EE had no significant effect on GSK3β expression levels in APPswe/PS1ΔE9 mice, regardless of housing conditions or brain regions (**Figure**
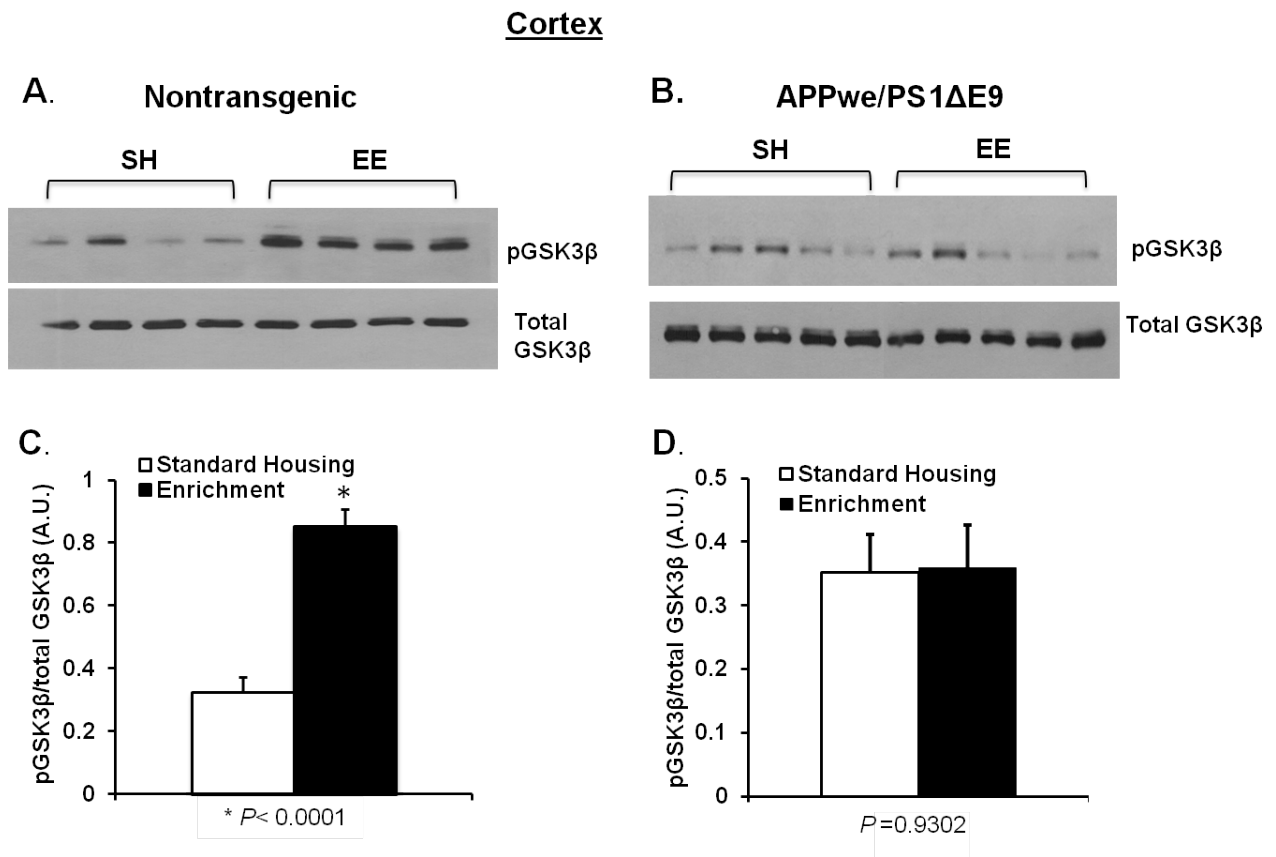
Cortex

FIGURE 23 Alterations in GSK3β expression level in the cortex of the nontransgenic and APPswe/PS1ΔE9 mice following EE.

Representative immunoblots of protein extracts from cortex of standard housing (SH) and enriched (EE) nontransgenic (**A**) and APPswe/PS1ΔE9 mice (**B**) for GSK3β expression. (**C, D**) Densitometric quantification of the Western blot was performed using Image J software, where pGSK3β serine 9 expression level was normalized to total GSK3β expression level. Data was presented as mean (arbitrary units) ± SE. *P<0.05, Student's t test, N=4 per group.
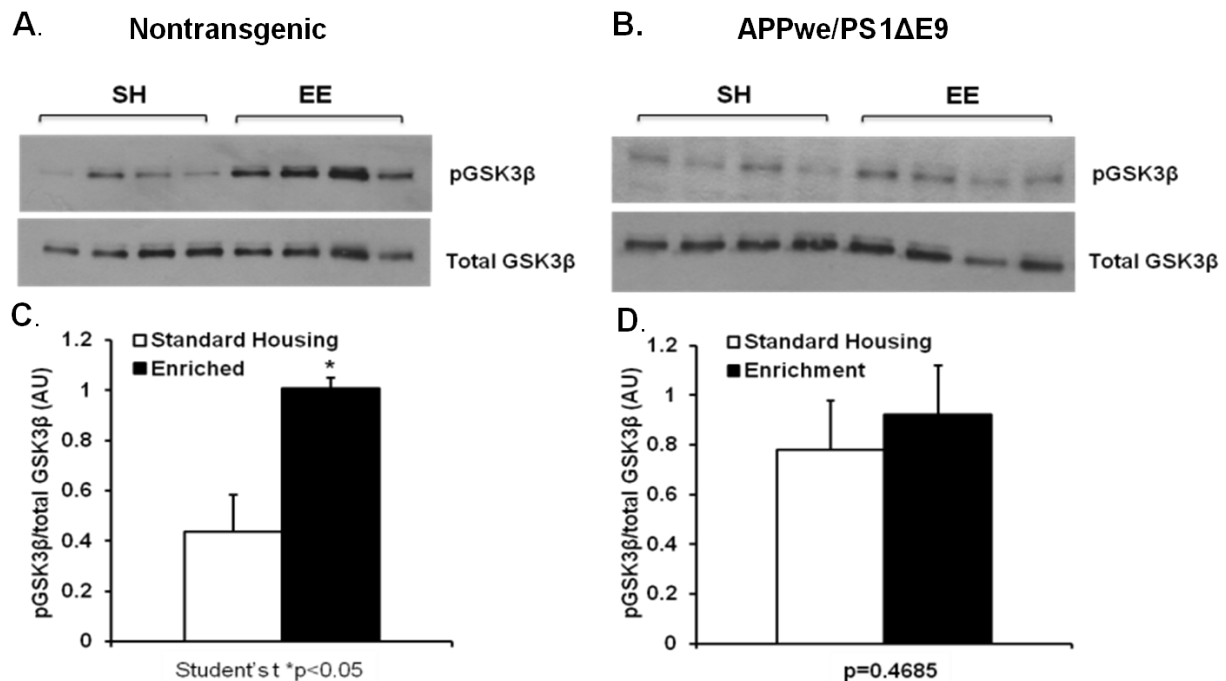
Hippocampus

FIGURE 24 Alterations in GSK3β expression level in the hippocampus of nontransgenic and APPswe/PS1ΔE9 mice following EE.

Representative immunoblots of protein extracts from hippocampus of standard housing (SH) and enriched (EE) nontransgenic (**A**) and APPswe/PS1ΔE9 mice (**B**) for GSK3β expression. (**C, D**) Densitometric quantification of the Western blot was performed using Image J software, where pGSK3β serine 9 expression level was normalized to total GSK3β expression level. Data was presented as mean (arbitrary units) ± SE. *$P<0.05$, Student's t test, N=4 per group.

23B, 24B). This may suggest that FAD-linked APPswe/PS1ΔE9 mutants compromise GSK3β level, and that EE fails to rescue this effect. Alternatively, this also suggests that EE is affecting the nontransgenic and APPswe/PS1ΔE9 mice via different molecular pathways.

4. **Downregulation of GSK3β expression level in the nontransgenic mice may be induced by the activation of its upstream PI3K/Akt signaling pathway.**

GSK3β is one of the major downstream substrates of Akt in the PI3K/Akt-dependent signaling pathway. Several studies have reported that physical exercise activates PI3K/Akt survival-promoting pathway, which in turn regulates GSK3β activities. This effect is thought to be mediated by an increased level of neurotrophic factors (Chen and Russo-Neustadt, 2005; Sakamoto et al., 2004). Based on our observation that implicated a reduced GSK3β activity following EE in the nontransgenic mice, we analyzed protein expression level of Akt in the cortex and hippocampus of standard housing and enriched mice (N= 5 per group) by Western blot analysis. Phosphorylation of Akt at Ser 437 activates Akt kinase activity. Higher expression level of pAkt indicates an increased activation of Akt. Western blots were analyzed for densitometric quantification using Image J software, normalizing pAkt expression level to the total Akt expression level. We observed that Akt expression level was upregulated in the cortex and hippocampus of nontransgenic mice experienced in enriched environment (**Figure 25A, 26A**), suggesting an increased Akt activity following EE. Similar to our previous findings with GSK3β, Akt activity in the cortex and hippocampus of APPswe/PS1ΔE9 mice was unaffected by environmental enrichment (**Figure 25B, 26B**). Quantitative analyses of immunoblots were shown in (**Figure 25C,D, 26C,D**).

Cortex

A. **Nontransgenic**

B. **APPwe/PS1ΔE9**
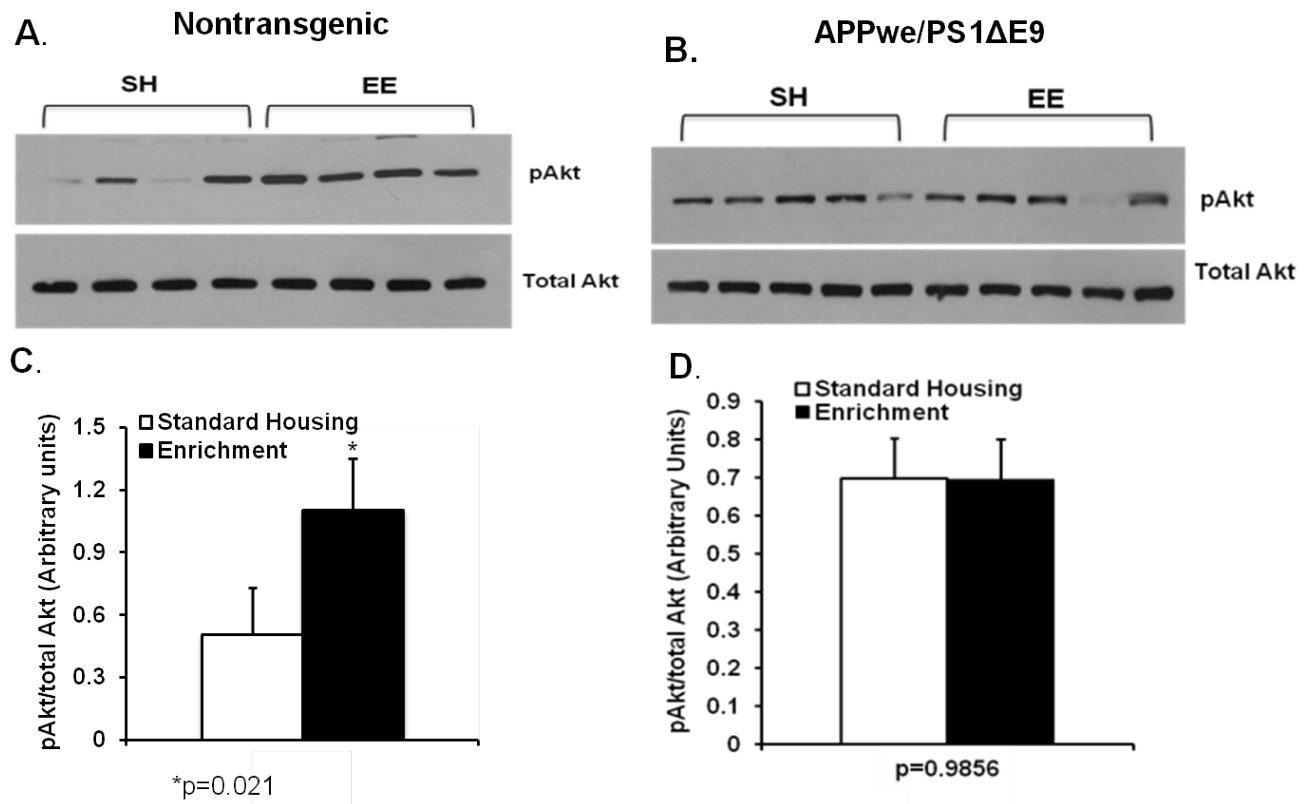
FIGURE 25 Alterations in Akt expression level in the cortex of nontransgenic and APPswe/PS1ΔE9 mice following EE.

Representative immunoblots of protein extracts from cortex of standard housing (SH) and enriched (EE) nontransgenic (**A**) and APPswe/PS1ΔE9 mice (**B**) for Akt expression level. (**C, D**) Densitometric quantification of the Western blot was performed using Image J software, where pAkt serine 437 expression level was normalized to total Akt expression level. Data was presented as mean (arbitrary units) ± SE. *$P<0.05$, Student's t test, N=4 per group.
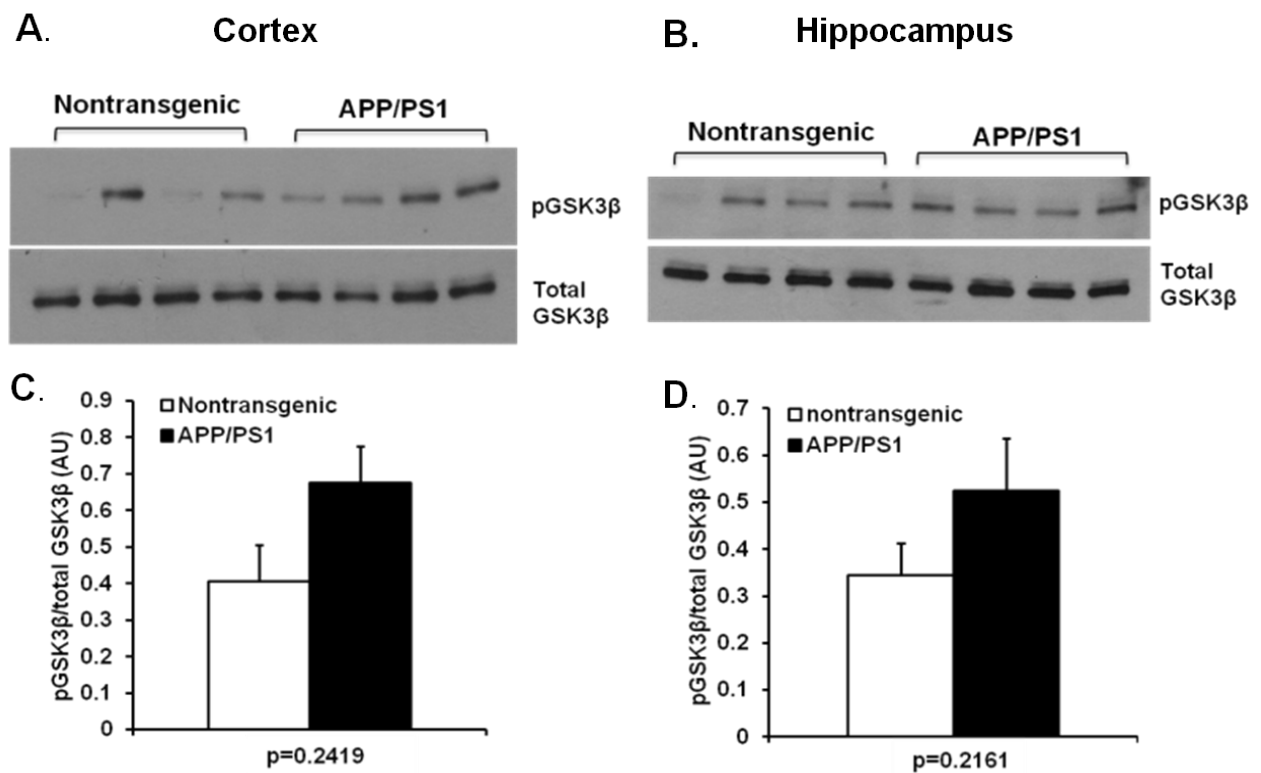
Hippocampus

A. **Nontransgenic**

B. **APPwe/PS1ΔE9**
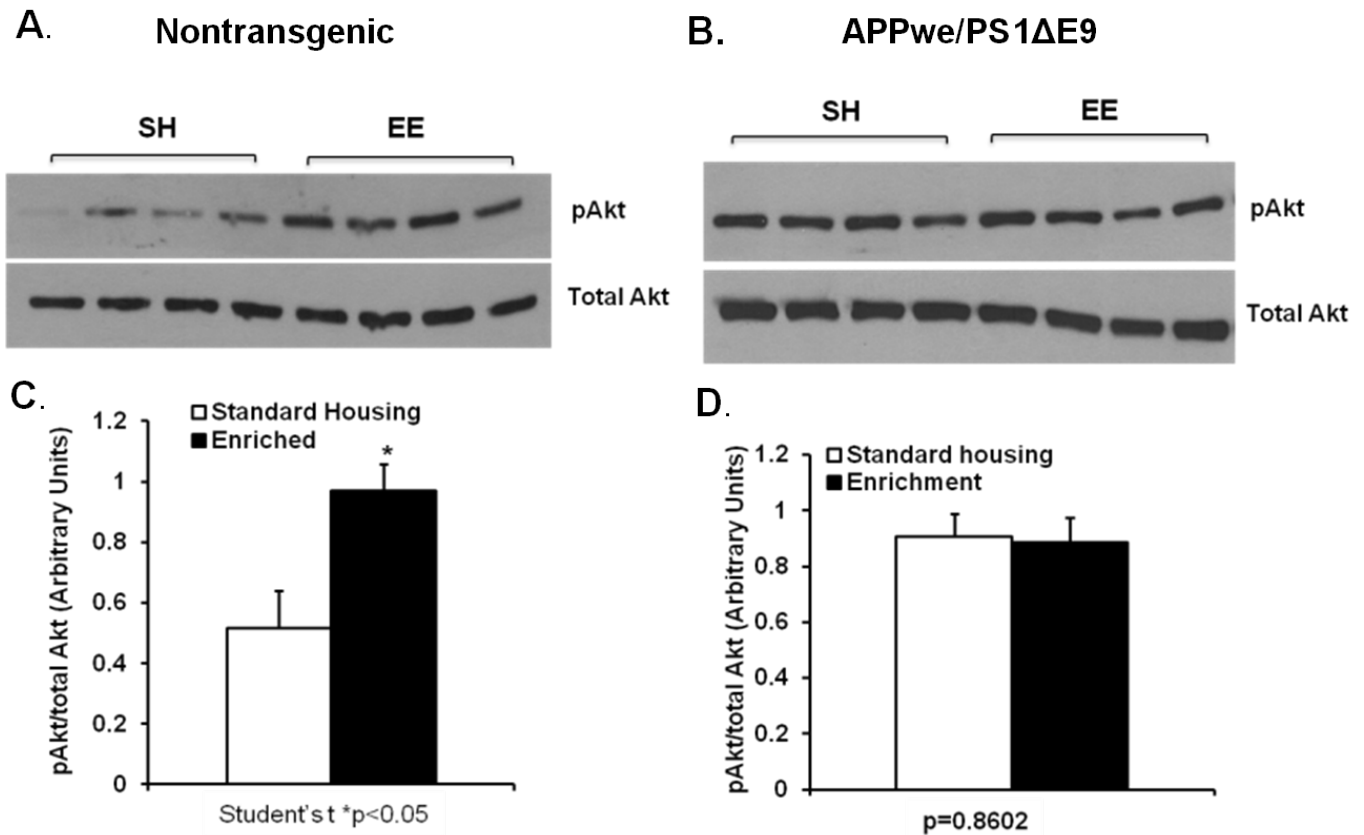
FIGURE 26 Alterations in Akt expression level in the hippocampus of nontransgenic and APPswe/PS1ΔE9 mice following EE.

Representative immunoblots of protein extracts from hippocampus of standard housing (SH) and enriched (EE) nontransgenic (**A**) and APPswe/PS1ΔE9 mice (**B**) for Akt expression level. (**C, D**) Densitometric quantification of the Western blot was performed using Image J software, where pAkt serine 437 expression level was normalized to total Akt expression level. Data was presented as mean (arbitrary units) ± SE. *$P<0.05$, Student's t test, N=4 per group.

5. **Expression level of protein kinase CK2 is significantly downregulated in the hippocampus of nontransgenic, but not in the APPswe/PS1∆E9 mice following EE.**

Casein kinase 2 (CK2) is an important kinase that has been implicated in AD and axonal transport due to its ability to phosphorylate Kinesin-1 and tau (Meggio and Pinna, 2003; Schafer et al., 2008). Not only can CK2 phosphorylate kinesin-1 and tau on its own, it is also a major priming kinase for GSK3β modification of KLC, such that GSK3β will not not phosphorylate KLC without a priming phosphorylation. Previously, we have shown that FAD-linked mice showed impairment in fast axonal transport and that environmental enrichment was able to enhance protein expression of Kinesin-1 in these mice ((Pigino et al., 2003), **Figure 11, 12**). To start to address the role of CK2 in FAD, we examined the expression level of CK2 in the hippocampus of nontransgenic and APPswe/PS1∆E9 mice maintained in standard housing condition (**Figure 27A**). Western blot result showed no significant difference in the level of CK2, suggesting that at 2 months of age, similar to what we observed earlier with GSK3β and Akt, FAD-linked mutations do not affect the endogenous activity level of CK2. However, CK2 activity has the tendency to change as a function of age and/or neuropathology in the brains of APPswe/PS1∆E9 mice (**Figure 20**).

Interestingly, in support of our previous observation concerning GSK3β and Akt, expression level of CK2 was significantly downregulated in the nontransgenic, but not in APPswe/PS1∆E9 mice experienced in enriched environment (**Figure 27C, 27E**). This further supports our speculation that either APPswe/PS1∆E9 mutants dominate the effect of EE on several signaling pathways or that EE affects nontransgenic and APPswe/PS1∆E9 mice via different molecular signaling pathway(s). Further experiments are required to examine the roles of CK2 in the context of Alzheimer's disease.

In summary, our data suggest that experience of wild type mice in an EE can effectively downregulate the activities of GSK3β and CK2, the two major kinases implicated in AD pathogenesis, possibly via the increase of BDNF level in the hippocampus that consequently activates PI3K/Akt signal transduction to promote neuroplasticity and deactivate kinases involved in AD pathology (**Table III**). In contrast, EE fails to modify GSK3β and CK2 expression levels in the APPswe/PS1ΔE9 mice, indicating that the effect of EE is not mediated through these kinases and that the effect of APPswe/PS1ΔE9 mutant variants may dominate the effect of EE on these kinase activities.
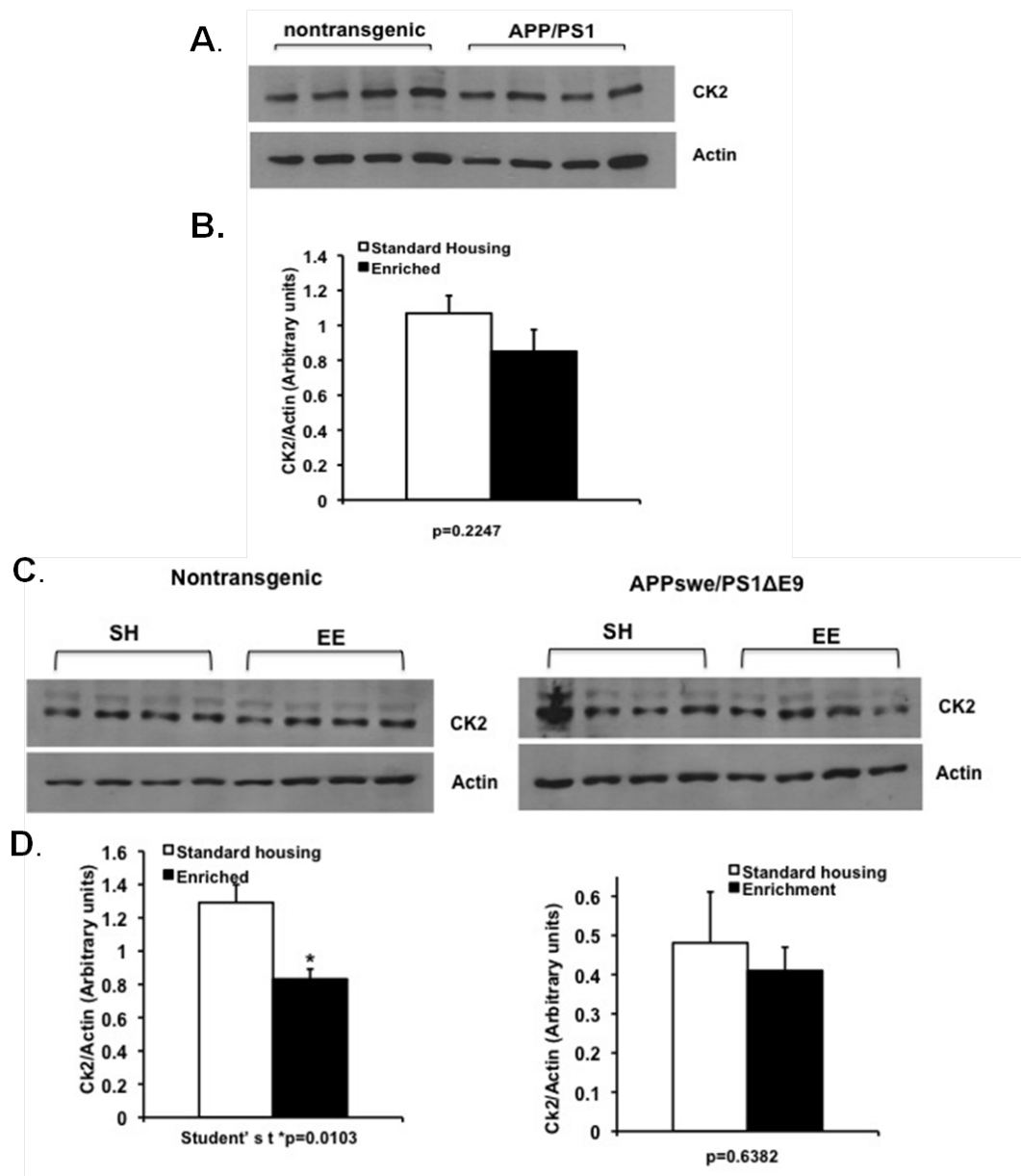
FIGURE 27 Alternation in CK2 expression following environmental enrichment.

(**A**) Representative immunoblots of protein extracts from cortex of 2-month old nontransgenic and APPswe/PS1ΔE9 mice housed in a standard laboratory condition and in an enriched environment (**C, E**). Mouse monoclonal CKIIα antibody was used to detect any changes in the expression level of CK2. (**B, D, F**) Densitometric quantification of the Western blots was performed using Image J software by normalizing CK2 expression level to actin expression level. Data was presented as mean (arbitrary units) ± SE. *$P<0.05$, Student's t test, N=4 per group.

TABLE III

ALTERATIONS IN PROTEIN EXPRESSION LEVELS FOLLOWING
ENVIRONMENTAL ENRICHMENT

Protein expression level	Targets	Nontransgenic vs. APPswe/PS1ΔE9	Nontransgenic SH vs. EE	APPswe/PS1ΔE9 SH vs. EE
Cortex	BDNF	↔	↔	↔
	GSK3β	↔	↓	↔
	Akt	↔	↓	↔
Hippocampus	BDNF	↔	↑	↑
	GSK3β	↔	↓	↔
	Akt	↔	↓	↔
	CK2	↔	↓	↔
	LTP	↔	↑	↑

↔ no change in expression

↑ increased expression following EE

↓ decreased expression following EE

V. DISCUSSION

A. Characterization of brain plasticity and AD pathology in young APPswe/PS1∆E9 mice, pre-onset of amyloid deposition.

1. <u>Neurogenesis is impaired early in APPswe/PS1∆E9 mice, and it may contribute to neuronal vulnerability in AD.</u>

In this study, we have shown that impaired neurogenesis is an early event in the development of AD pathology, and it may contribute to functional and behavioral deficits in AD, such as the progression loss of hippocampus- and olfaction-dependent learning and memory. In addition, we have presented evidence that tau hyperphosphorylation underlies, at least in part, impaired neurogenesis in the brains of APPswe/PS1∆E9 mice. This study also provides evidence that tau hyperphosphorylation is prominent in NPCs of these mice.

The role of adult neurogenesis has been extensively studied in the past few decades, providing evidence for its critical role in the maintenance of structural and functional integrity of the brain, and its therapeutic potentials for brain repair and cognitive improvement. The preservation of NSCs and NPCs sources in the postnatal brain is increasingly recognized to play critical roles in both the olfaction system and in the hippocampus, as newly formed neurons are incorporated in the olfactory bulb and granule layer of the dentate gyrus respectively, and they are thought to play a role in numerous aspects of learning and memory (Imayoshi et al., 2008; Zhao et al., 2008b). Thus impairments in these processes at a very early stage of AD, may cause or contribute to dysfunction of the olfaction system and hippocampus, leading to deficits in olfaction and hippocampus-dependent learning and memory.

Numerous studies have suggested that the extents of neurogenesis in the SVZ and the DG are declined with age, raising the possibility that reduced neurogenesis may

account, at least in part, for impaired learning and memory and cognitive deterioration in the elderly (Kempermann et al., 2002; Kuhn et al., 1996; Seki and Arai, 1995; Tropepe et al., 1997). There is still very little known about status of neurogenesis in patients affected with AD and in individuals with mild cognitive impairments (MCI). The examination of neurogenesis in post-mortem brain provides very little information about the dynamic process that is responsive to age, disease stage and environmental cues. Examination of neurogenesis in brain tissue of AD patients revealed increased expression of immature neuronal marker proteins (Jin et al., 2004b). However, these observations have been challenged recently, where new evidence suggests that in the aging and AD brains, there is a significant decline in the extents of proliferation of progenitor cells and the number of new neurons (Boekhoorn et al., 2006; Brinton and Wang, 2006). Taken together with the lack of means to detect neurogenesis in living individuals, information about neurogenesis in humans and in AD patients is scarce.

As current research on Alzheimer's disease relies heavily on the use of AD mouse models, they have become important tools for the temporal and spatial analyses of neurogenesis in relation to AD disease progression in the postnatal brains. The mechanism by which deficits in neurogenesis in AD take place is yet to be determined. In addition, it is not fully understood how the extent of neurogenesis changes in response to events such as amyloid deposition, neurofibrillary tangles and neuronal loss. Studies of neurogenesis in FAD models, particularly in APPswe/PS1ΔE9 mice, were conducted for the most part after the onset of amyloid deposition. Present evidence seems to suggest that altered neurogenesis in AD is a side effect rather than a cause or contributing factor to AD pathogenesis. Studies by two groups examined the extents of cell proliferation and newly differentiating cells in the SGL of 5,6 and 9 month-old APPswe/PS1ΔE9 mice, where they

found significant reductions in cell proliferation and neuronal differentiation in 9 month-old mice, suggesting that other factors apart from amyloid deposition may induce these alterations. This data also suggests that the status of neurogenesis is a function of age-specific neuropathology (Li et al., 2008; Taniuchi et al., 2007). In addition, study by the same research group also examined the number of proliferating cell nuclear antigen (PCNA)-positive cells in the SGL and the SVZ of APPswe/PS1ΔE9 mice at the age of 9 months and found no difference in the number of PCNA-positive cells in these two areas compared to nontransgenic mice (Niidome et al., 2008). In contrast, they observed a significant reduction in the number of BrdU-positive cells in the SGL but not in the SVZ of APPswe/PS1ΔE9 mice.

The significance of our study is to address the alterations in neurogenesis pre-onset of amyloid deposition in APPswe/PS1ΔE9 mice. We reason that impaired neurogenesis is not a consequence of amyloidosis, but instead it acts as an important contributing factor in AD pathogenesis. Hence, we examined whether alterations in neurogenesis are an early event that can potentially play a role in AD pathogenesis. Because APPswe/PS1ΔE9 mice start to exhibit amyloid deposition around 4-5 months of age, in this study we examined neurogenesis pre-onset of amyloid deposition at 2 months of age. We have shown for the first time that the proliferation and early differentiation of neural progenitor cells are severely impaired in the SVZ and SGL of these mice as early as 2 months of age, long before the appearance of amyloid deposition or memory impairments (**Figure 3, 4**). This suggests that amyloid deposition is not a primary cause for impaired neurogenesis in these mice and that impaired neurogenesis may underlie or exacerbate memory impairments and neuronal vulnerability in these brain regions.

2. <u>Tau hyperphosphorylation is a prominent event in the neurogenic niches of APPswe/PS1ΔE9 mice.</u>

In this study, we also examined the significant role of tau hyperphosphorylation in impaired neurogenesis in APPswe/PS1ΔE9 mice. We found a striking increase in the levels of tau phosphorylation in the SVZ and the SGL of these mice, suggesting that tau hyperphosphorylation may play a critical role in impaired neurogenesis in our AD transgenic mice. Hyperphosphorylation of tau in AD is thought to be the primary event that induces detachment of tau from microtubules (Bramblett et al., 1993), which leads to filament formation and subsequently the accumulation of intracellular neurofibrillary tangles. It is, however, important to acknowledge that, while the degree of cognitive impairment is not well-correlated with the extent of amyloid deposition, the extent of neurofibrillary tangles is very well-correlated with the degree of cognitive decline in Alzheimer's patients (Arriagada et al., 1992; Ghoshal et al., 2002; Mitchell et al., 2002). Phosphorylation of tau occurs early in the pathogenesis of neurofibrillary tangle formation (Biernat et al., 1992). Several phosphorylation sites on tau protein modulate the binding to microtubules, therefore hyperphosphorylation at these sites may decrease the affinity of tau for microtubules, leading to microtubules depolymerization (Bramblett et al., 1993; Lindwall and Cole, 1984). Previous studies have shown that hyperphosphorylation of tau around dystrophic neurites resembled paired helical filaments (PHFs) in the cortex and the hippocampus of APPswe/PS1m146L mice at 8 months of age (Kurt et al., 2003). However, It is still not clear what is the functional role of tau hyperphosphorylation in relation to neurogenesis in AD. Here, we aim at examining the role played by tau phosphorylation in the neurogenic regions of APPswe/PS1ΔE9 mice pre-onset of amyloid deposition.

Functional implications of tau phosphorylation are far-reaching when it comes to growth and development of NPCs, cell mitosis, axonal transport, neuronal process elongation, and neuronal maturation (for review see (Johnson and Stoothoff, 2004). We observed that tau-5 immunoreactivity was co-localized with BrdU, GFAP, and DCX in APPswe/PS1ΔE9 mice (Demars et al., 2010), in addition to high level of tau phosphorylation immunoreactivity in the neurogenic regions and NPCs derived from these mice (**Figure 5, 6**). This data suggests that alteration in tau phosphorylation may be detrimental to the development of NSCs, NPCs, and neuroblasts. Interestingly, a recent evidence suggests that hyperphosphorylation of tau can lead to chromosome aberrations and subsequently mitosis obstruction (Rossi et al., 2008). This may imply that reduced proliferation of the progenitor cells, as was observed *in vivo* and *in vitro* in APPswe/PS1ΔE9 mice, may be due partially to hyperphosphorylation of tau in these neural progenitors. Moreover, several kinases and phosphatases, such as glycogen synthase kinase 3 (GSK3) and casein kinase 2 (CK2), play important roles in tau phosphorylation in AD [(Morfini et al., 2002b; Wang et al., 1998), for review see (Trojanowski and Lee, 1994, 1995)]. PHF-1 antibody used in our studies recognized phosphorylated epitopes at Ser-396 and Ser-404 of tau (Hernandez et al., 2003; Seubert et al., 1995), the epitopes that were thought to be preferentially phosphorylated by GSK3β (Godemann et al., 1999; Lee et al., 2003). Our data suggests that alterations in these enzymatic activities may take place in the neurogenic niches of APPswe/PS1ΔE9 mice, and it may underlie tau hyperphosphorylation in these brain areas. GSK3β is a component of the Wnt signaling pathway, which plays critical roles in adult neurogenesis (Lie et al., 2005), microtubule dynamics (Frame and Cohen, 2001), and fast axonal transport (Morfini et al., 2002b). GSK3β is inactivated by phosphorylation at serine 9 (Ser 9) at its N'-terminus. Numerous well-known substrates of GSK3β include PS1, β-

catenin, tau, and kinesin-1 light chains (KLC) (Morfini et al., 2002b; Takashima et al., 1998; Tesco and Tanzi, 2000). Importantly, GSK3β and CK2 have been shown to inhibit fast axonal transport (Morfini et al., 2002a; Morfini et al., 2002b). In this regard, phosphorylation of KLC by GSK3β promotes the release of kinesin-1 from membrane-bound organelles, which in turn leads to a reduction in fast anterograde axonal transport (Morfini et al., 2002a). Taken together, these pieces of evidence raise the likelihood that axonal transport may be dysfunctional in newly formed neuroblasts of APPswe/PS1ΔE9 mice, a hypothesis that is supported by our observation that PHF-1 immunoreactivity is pronounced in neuroblasts of APPswe/PS1ΔE9 mice (Demars et al., 2010).

Evidence in this first part of our study suggests that neural progenitor cells in both SVZ and SGL are affected early and severely, leading to impaired neurogenesis in the APPswe/PS1ΔE9 mice. Expression of FAD mutations induces intrinsic deficits in NPCs, as well as deficits in their neurogenic microenvironments. It is reasonable to assume that these impairments in neurogenesis may contribute to the hippocampal and olfactory dysfunction, as well as to the neuronal vulnerability in AD.

B. Environmental enrichment rescues impaired neurogenesis, enhances synaptic plasticity and attenuates neuropathology in FAD-linked APPswe/PS1ΔE9 mice.

In this second part of the study, we examined the hypothesis that brain plasticity can be induced by environmental factors in FAD-linked APPswe/PS1ΔE9 transgenic mice, and resulted in reducing neuropathology and rescuing brain structure and function. Although a lot of studies have examined the effect of enriched environmental conditions on AD neuropathology (data is summarized in **Table I**), the controversial observations in these studies called for further examination of the effect of the experience in a complex environment on amyloid metabolism and tau post-translational modifications. Investigations

have been focusing on looking at the effect of EE on the extent of amyloid deposition, which is the "end product" of amyloid pathology, whereas the effect of EE on oligomeric Aβ or on tau phosphorylation is still uncertain. Billings and colleagues have shown previously that involuntary repeated spatial water-maze training decreased amyloid and tau pathologies in 3xTg-AD mice (Billings et al., 2007). Whether or not voluntary experience in an enriched environmental conditions can exert similar effects is not known. Likewise, the effect of EE on other critical aspects of hippocampal plasticity in FAD mice is still poorly characterized. Our study is aimed at addressing these issues, by adding novel insights into the effect of the experience in a complex environment on hippocampal plasticity in FAD mice.

Equally important is the examination of the effect of EE in young FAD mice with neurogenic impairments, when they are lacked of amyloid deposition, inflammation, neuronal degeneration that occur later in life and may cause secondary alterations in neurogenesis. We have shown earlier that impairments in neurogenesis at this stage are the direct result of the expression of mutants APP and PS1. Unfortunately, very little information is available on the status of neurogenesis pre-onset of amyloid deposition, and it is not known whether EE can rescue neurogenic impairments at this stage. Moreover, understanding the molecular mechanisms of the changes in neurogenesis both at the initial and at the later stages of AD will contribute greatly to the development of AD biomarkers and therapeutics. Our study has demonstrated that the experience of APPswe/PS1ΔE9 mice in enriched environmental conditions significantly reduced the levels of oligomeric Aβ accumulation and abnormal tau hyperphosphorylation (the neurotoxic precursors that constitute AD hallmarks), This suggests that experience in an enriched environment can overcome compromised neurogenesis and enhances neuronal function, as manifested by the increased expression of critical axonal transport players, as well as the enhancement of hippocampal LTP expression.

1. **Environmental enrichment rescues impaired hippocampal neurogenesis in young APPswe/PS1∆E9 mice.**

Experience in EE induces neurogenesis in the SGL of the DG, but not in the SVZ (Kempermann et al., 1997; van Praag et al., 1999b), probably due to the nature of the stimuli provided by EE. Here we have demonstrated that stimuli provided by EE can successfully reverse impaired neurogenesis in the SGL of APPswe/PS1∆E9 mice early in the pathogenesis of the disease by promoting NPCs proliferation, neuronal differentiation, selective astrocyte differentiation, and neuronal maturation (**Figure 7, 8, 9**). Previously, Choi and colleagues have shown that the experience of FAD-linked mice harboring PS1 variants in an enriched environment enhanced neurogenesis only in mice expressing PS1 human wild type and not in mice expressing mutant PS1∆E9 or PS1M146L (Choi et al., 2008). Unlike in APPswe/PS1∆E9 mice, hippocampal neurogenesis is not impaired in the PS1∆E9 or PS1M146L mice to begin with. Therefore, the neurogenic mechanisms activated in these two studies may be significantly different. In addition, PS1∆E9 and PS1M146L transgenic mice have significantly lower levels of Aβ than APPswe/PS1∆E9 mice, and they do not exhibit amyloid deposition later in life (Lazarov et al., 2005b).

It has become apparent that both APP and PS1 play important roles in adult neurogenesis by regulating the proliferation, differentiation and survival of the neural stem/progenitor cells. In addition, increasing evidence has suggested that dysregulation or dysfunction of these molecules may compromise these processes and resulted in an impaired neurogenesis [for review, see (Lazarov and Marr, 2010)]. PS1 has been considered as an appealing signaling molecule in many fundamental processes, such as Notch signaling, Wnt/β-catenin signaling, E-cadherin signaling and ErbB-4-mediated signaling (De Strooper et al., 1999; Lie et al., 2005; Sardi et al., 2006; Tesco et al., 1998).

Studies in transgenic mice harboring FAD-linked mutant PS1 reveal deficits in hippocampal neurogenesis (Chevallier et al., 2005; Feng et al., 2001; Haughey et al., 2002a; Wang et al., 2004; Wen et al., 2004; Zhang et al., 2007). In this study, by the examination of isolated neurospheres, we have determined that APPswe/PS1ΔE9 mutations intrinsically induce tau hyperphosphorylation and impaired the proliferation of NPCs in culture (**Figure 6**). Given our observations showing the rescue of impaired hippocampal neurogenesis after experience in an enriched environment, it is reasonable to assume that molecular signals provided by EE overcome the pathological effects that mutant APP and PS1 exert on neurogenesis. Indeed, concomitant to enhanced neurogenesis, the experience of APPswe/PS1ΔE9 mice in an enriched environment significantly reduced the levels of oligomeric Aβ and tau hyperphosphorylation.

2. **Environmental enrichment ameliorates amyloid pathology in APPswe/PS1ΔE9 mice.**

Previously, we have shown that EE effectively reduced the extent of amyloid deposition in the cortex and the hippocampus of 6 months-old APPswe/PS1ΔE9 mice (Lazarov et al., 2005b). The amyloid deposits are composed of insoluble extracellular aggregates of the amyloid-β peptides. For a long time, it was generally accepted that the insoluble amyloid plaques were the key player underlying cognitive deterioration in AD. Until in the late 1990s, increasing evidence in AD research field called for the attentions of the soluble, nonfibrillar form of Aβ, which has been shown to significantly correlate with the extent of synaptic loss and severity of cognitive impairments (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). Soluble oligomeric Aβ peptides of $Aβ_{42}$ have been shown to decrease dendritic spine density and to potentially induce hyperphosphorylation of tau. It has also been shown to inhibit LTP and to facilitate long-term depression (LTD) in the

hippocampus (Li et al., 2009; Shankar et al., 2008). It is now well-accepted that the toxic form of Aβ is in the soluble oligomeric conformation.

Nevertheless, it is not known whether EE plays any effects on soluble forms of Aβ. Using conformation-specific antibody, our study is the first one to demonstrate that EE can significantly reduce the neurotoxic oligomers of Aβ that primarily accumulate in the hippocampus and cortex (**Figure 10**). A few reports have examined the impact of EE on Aβ levels and amyloid deposition in transgenic mice, revealing a variable effect of EE on these processes (**see Table I**). Detailed examination of these studies reveals critical differences in experimental design, such as the composition of the enriched environment, accessibility to running wheels, duration of differential experience, treatment relative to onset of deposition, age, gender, type of transgenic mice, and etc. [for detailed analysis of these studies and a comparative description, see (Lazarov and Larson, 2007)]. It should be noted that in most FAD transgenic mice used in these studies, there is an age-dependent increase in amyloid deposits. The extent of amyloidosis, its quantification, and the cause-and-effect pathological interpretation might be challenging as animals get older and as the secondary effects (e.g. inflammation) become significant. The current study was designed to avoid any complications that might result from these mechanisms, by using young animals before the onset of amyloid deposition. Our results strongly support the conclusion that the experience of APPswe/PS1ΔE9 mice in an enriched environment reduced steady-state levels of soluble and insoluble Aβ. The mechanism by which EE reduces levels of Aβ is still unknown. We previously reported that mice experienced in EE exhibit higher level of neprilysin (NEP), an amyloid β-degrading enzyme, suggesting that EE may reduce levels of Aβ by promoting degradation and clearance of the amyloid peptides (Lazarov et al., 2005b). In this study, our results further suggest that one of the mechanisms by which EE downregulates the level of

amyloid deposition is by reducing the level of oligomeric Aβ, the precursor of amyloid deposition.

In addition, Lazarov and colleagues have identified an array of genes that are being modulated in the hippocampus and the cortex of APPswe/PS1ΔE9 mice after experience in an enriched environment. Intriguingly, these genes play important roles in the neuronal survival, neurogenesis, and amyloid metabolism (Lazarov et al., 2005b). Our results in this study are in agreement with these observations. We have shown that while steady-state levels of full-length APP did not change, the levels of oligomeric Aβ are indeed downregulated following EE. This suggests that the experience in an enriched environment modulates Aβ clearance and degradation rather than APP production.

3. <u>Environmental enrichment ameliorates tau pathology in APPswe/PS1ΔE9 mice.</u>

In this study we have shown that experience in an enriched environment also attenuates hyperphosphorylation of tau, another important neuropathological hallmark of AD. We have shown previously that tau is hyperphosphorylated in both neurogenic regions of the APPswe/PS1ΔE9 brains. Nevertheless, whether or not EE has any effects on tau phosphorylation has never been studied. One study using 3xTg-AD model has shown that involuntary repeated spatial water-maze training significantly decreased Aβ*56 and tau hyperphosphorylation, using AT8, AT180 and AT270 antibodies (Billings et al., 2007). However, the difference between voluntary exploration in EE versus involuntary training exercise in this study should be noted. Using EE paradigm, we showed that PHF-1 immunoreactivity, an indicative of tau phosphorylation at the epitopes that are clinically relevant to AD, was dramatically reduced in the brains of APPswe/PS1ΔE9 mice after experience in an enriched environment (**Figure 10, 11**). Several kinases that are known to cause hyperphosphorylation of tau, such as GSK3β and CK2, are also shown to inhibit fast

axonal transport. This led us to examine whether the markers of axonal transport were altered following EE. Our data has shown that indeed the expression of kinesin-1, a major motor of anterograde axonal transport in neurons, was increased in the brains of these mice following EE. In fact, a very recent study confirmed our finding by showing BDNF-mediated upregulation of kinesin superfamily motor protein 1A (KIF1A) in the hippocampi of mice experience in enriched environment for as little as 2 weeks. In the same study, they also showed that the administration of BDNF in the primary hippocampal culture also increased the levels of KIF1A and KIF1A-mediated cargo transport, in addition to induce hippocampal synaptogenesis (Kondo et al., 2012). Furthermore, the reduction of oligomeric Aβ can also have a positive impact on axonal function and transport. In support of this notion, the perfusion of oligomeric Aβ (but not fibrillar or unaggregated Aβ) dramatically inhibits both directions of axonal transport (Pigino et al., 2009). The increase in neuronal impulse activity has long been known to affect fast axonal transport (Antonian et al., 1987; Hammerschlag and Bobinski, 1992), as well as the synthesis of neuronal proteins, which raises the possibility that the enhanced delivery of synaptic components by fast anterograde axonal transport may contribute to the attenuation of pathological aspects of AD following the exposure to an enriched environment.

The current study adds another novel aspect to the mechanism underlying enrichment-induced reduction in neuropathology by showing that the experience of APPswe/PS1ΔE9 mice in an enriched environment modulates phosphotransferase activities. Hyperphosphorylation of tau reflects elevated neuronal kinase activities in AD brains (LaPointe et al., 2009; Lee and Trojanowski, 2006; Pigino et al., 2009; Pigino et al., 2003). Downregulation of these processes suggests that the kinase and phosphatase signalings are modulated after experience in an enriched environment. In fact, as discussed below,

this study suggests that this might be one of the mechanisms by which EE attenuates AD pathology.

4. <u>Environmental enrichment enhanced synaptic plasticity in APPswe/PS1ΔE9 mice.</u>

Increasing evidence suggests that oligomers of Aβ peptide, but not the monomeric forms, can potentially disrupt LTP and cognitive functions (Cleary et al., 2005; Townsend et al., 2006; Walsh et al., 2002). Our electrophysiological studies of nontransgenic and APPswe/PS1ΔE9 mice following experience in EE yielded three interesting results. First, no differences in input-output curves or paired-pulse facilitation were attributable to genotype or enrichment. Second, LTP was not significantly reduced in the FAD-linked transgenic mice housed under standard conditions at early ages. As noted above, these mice were tested at a relatively young age, and before the development of amyloid plaques. Nevertheless, the transgenic mice did have measurable levels of oligomeric Aβ and reduced neurogenesis in the DG. These effects are apparently insufficient to interfere with synaptic transmission and LTP in the CA1 field. Third, EE enhanced LTP in both APPswe/PS1ΔE9 and control mice. Our studies provide the first evidence that experience in EE enhances LTP in FAD-linked transgenic mice, although a similar effect was noted previously in wild-type mice (Duffy et al., 2001). The mechanism underlying LTP enhancement is not known but could involve EE-stimulated production of BDNF (Falkenberg et al., 1992; Kramár et al., 2004; Pang et al., 2004), which is being upregulated in APPswe/PS1ΔE9 mice after enrichment [(Lazarov et al., 2005b), **Figure 15, 17**]. BDNF may enhance hippocampal LTP by regulating the endocytosis of NMDA receptors from the plasma membrane (Farmer et al., 2004; Snyder et al., 2005), by enhancing neurogenesis (Snyder et al., 2001 ; van Praag et al., 1999b), or perhaps by enhancing axonal transport.

Numerous studies have shown that FAD-linked transgenic mice with amyloid pathology exhibit impairments in learning and memory tasks, including acquisition of long-term spatial memory (Chapman et al., 1999; Chishti et al., 2001; Dewachter et al., 2002; Dineley et al., 2002; Jankowsky et al., 2005), spatial reversal learning (Saura et al., 2005), use of spatial working memory (Arendash et al., 2001; Morgan et al., 2000; Trinchese et al., 2004), acquisition of social recognition memory (Ohno et al., 2004), object recognition memory (Dewachter et al., 2002), and contextual fear conditioning (Dineley et al., 2002). In some cases, the severity of the impairment has been correlated to Aβ levels in the brains of individual mice (Arendash et al., 2001; Gordon et al., 2001; Puolivali et al., 2002), and treatments aimed to reduce Aβ levels (e.g. immunization with Aβ or Aβ antibodies) can reverse these learning deficits. Most of these tasks depend on the functions of the hippocampus, therefore the deficits in neurogenesis or hippocampal synaptic plasticity (i.e. LTP) are possible causes for the learning deficits in these transgenic mice.

Although studies have suggested that the experience of FAD mice in an enriched environment attenuates cognitive deficits, the mechanisms underlying this improvement remain controversial and largely unknown. Our study suggests a few possible mechanisms. One possible explanation that derives from our work is that enhanced cognition after EE may result in improved LTP, rescued neurogenesis, or both. This is consistent with the recent data showing that the intracellular oligomeric Aβ blocks synaptic transmission when directly injected in the presynaptic terminals (Moreno et al., 2009). In addition, increasing evidence suggests that the newly formed neurons that integrate into the granular layer of the DG may play a role in the hippocampus-dependent functions [for reviews, see (Aimone et al., 2006; Zhao et al., 2008a)]. Rescue of the impaired neurogenic phenotype in APPswe/PS1ΔE9 mice by EE may underlie, at least in part, its effect on LTP and cognitive function in these mice. Finally, the enhanced axonal transport concomitantly with the

reduced levels of both oligomeric Aβ and hyperphosphorylated tau may contribute to the improved neuronal function and the enhanced cognition.

C. Molecular mechanisms mediating the effect of environmental enrichment on brain plasticity and AD pathology.

In spite of the extensive use of EE as an experimental paradigm for potential therapeutic method for neurodegenerative diseases and aging, the mechanistic molecular signaling pathway(s) underlying its effects is never fully elucidated. This is particularly true when it comes to the explanation for the effects of EE on alleviating AD-related deficits. The outcome of EE is most certainly involved multiple signaling pathways that potentially interact with one another and result in various beneficial effects that we observed experimentally. Recently, increasing attentions have been paid to understand the effects of experience-dependent epigenetic modifications that tightly link the interactions between the regulation of gene expression and the environments. Recent studies suggest that environmental enrichment improves spatial memory and restores the capacity to form memory via increasing the level of histone acetylation in the hippocampus. This effect can also be mimicked using HDAC inhibitors [(Fischer et al., 2007), for review see (Sweatt, 2009)]. In addition, EE has been shown to modulate neuroplasticity by regulating energy metabolism, reducing oxidative cell stress and by stabilizing cellular calcium homeostasis in animal model of aging (Mattson et al., 2002; Mattson et al., 2001; Mattson et al., 2000).

1. <u>Environmental enrichment enhances production of BDNF in the hippocampus of nontransgenic and APPswe/PS1∆E9 mice.</u>

Numerous studies support the role of EE-induced neurotrophic factors in promoting neuronal survival and function. EE has shown to upregulate gene transcription encoding neuronal proteins that are important for promoting neuronal growth/structure, neuronal

survival (Rampon et al., 2000; Stranahan et al., 2010) and prevent spontaneous apoptosis (Young et al., 1999). The most predominant one is the upregulation of BDNF following EE and physical exercise (Adlard and Cotman, 2004; Adlard et al., 2005a; Falkenberg et al., 1992; Nithianantharajah and Hannan, 2006; Oliff et al., 1998; Pham et al., 1999; Zajac et al., 2009). Others, such as nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3) and insulin-like growth factor 1 (IGF-1) have also been reported to increase following EE (Falkenberg et al., 1992; Mattson et al., 2004b; Pham et al., 1999; Pham et al., 2002; Torasdotter et al., 1996, 1998). Increased BDNF levels are associated with enhanced activity of cyclic AMP response element binding protein (CREB) (Shieh and Ghosh, 1999). BDNF-TrkB signaling modulates neuronal differentiation, neural survival and synaptic plasticity via multiple signaling pathways, i.e. extracellular signal regulated kinases (ERKs), PI3K/Akt and PLCγ/PKC signaling pathways, respectively (Ohira and Hayashi, 2009). Several studies have reported that physical exercise induces hippocampal BDNF level, thereby activating the PI3K pathway and sequentially regulating Akt and GSK3β activities in the skeletal muscles and in the hippocampus (Bruel-Jungerman et al., 2009; Chen and Russo-Neustadt, 2005; Sakamoto et al., 2004). BDNF can also modulate synaptic plasticity via activation of mitogen-activated protein kinase kinases (MEKs) and ERKs (Bramham and Messaoudi, 2005; Kang et al., 1996). More importantly, there is a close connection between BDNF, LTP and oligomeric Aβ. BDNF has been shown to regulate postsynaptic late-phase LTP in the hippocampus of normal and aging mice (Kovalchuk et al., 2002; Pang and Lu, 2004). Not only can oligomeric Aβ disrupt LTP, it can also decrease basal level of BDNF mRNA level via specific downregulation of BDNF transcripts IV and V (Garzon and Fahnestock, 2007).

In AD, although many studies have shown that the experience of FAD-linked transgenic mice in EE enhances neurogenesis and improves cognitive functions, the molecular mechanism(s) underlying the effects of EE on neurogenesis, AD pathology and cognitive improvement is still largely unclear. Alterations in BDNF levels are observed in the cortex and hippocampus of Alzheimer's disease patients (Murer et al., 2001; Narisawa-Saito et al., 1996; Phillips et al., 1991), and BDNF immunoreactivity is greatly associated with senile plaques (Ferrer et al., 1999; Murer et al., 1999). However, evidence to date has been conflicting and BDNF has been reported to increase (Durany et al., 2000), as well as decreased in the hippocampus of post-mortem brains of the Alzheimer's patients (Conner et al., 1997; Hock et al., 2000; Michalski and Fahnestock, 2003). Interestingly, BDNF has also been shown to induce rapid dephosphorylation of tau protein through PI3K/Akt signaling pathways (Elliott et al., 2005). Collectively, these findings suggest that the reduced levels of BDNF in AD may exacerbate tau hyperphosphorylation, which could contribute to the synaptic dysfunction and neuronal degeneration in AD patients. Both environmental enrichment and physical exercise have been shown to effectively induce hippocampal BDNF in brain regions of many AD mouse models (Adlard and Cotman, 2004; Adlard et al., 2005a; Adlard et al., 2004; Blurton-Jones et al., 2009; Mattson et al., 2004a; O'Callaghan et al., 2009; Wolf et al., 2006). Previously, we have demonstrated that BDNF gene expression is upregulated in APPswe/PS1ΔE9 mice following EE by microarray analysis (Lazarov et al., 2005b). In this study, we examined the protein levels of total BDNF and reconfirmed the mRNA levels of BDNF in APPswe/PS1ΔE9 and nontransgenic mice following EE. In agreement with the earlier findings, we observed a significant increase in BDNF, both protein and mRNA levels (**Figure 15, 17**), in the hippocampus of APPswe/PS1ΔE9 and nontransgenic mice that experienced in an enriched environment. The increase of BDNF at

the protein level is only specific to the hippocampus and not in the cortical regions of these mice, further supports previous evidence that the effect of EE is brain-regions specific, where it only affects neurogenesis in the SGL and not SVZ. It is also noteworthy to mention that mRNA expression level of overall neurotrophic factors in APPswe/PS1ΔE9 is not impaired at 2 months of age (**Figure 16**). It strongly suggests that early impairment in neurogenesis in these mice is not a direct consequence of the lack of neurotrophic factors to support neural progenitor cells and neurogenic niches, but rather it is resulted from the intrinsic effects of APP and PS1 mutants. Interestingly, supporting evidence from previous reports, our study also found significant upregulation of NGF mRNA level in all mice experienced in an enriched environment, indicating the roles of NGF in EE-induced plasticity and synaptic functions (Mohammed et al., 1993; Olsson et al., 1994). In contrast to Wolf and colleagues' study that showed upregulation of NT-3 in AD mice following EE (Wolf et al., 2006), our study only observed the upregulation of NT-3 mRNA level in the nontransgenic and not in the APPswe/PS1ΔE9 mice following EE (**Figure 17**). It is still unclear what causes this discrepancy. One explanation could be the use of different AD mouse models and the use of different EE experimental protocols.

Previous studies have shown that living in social isolation decreased BDNF levels in the forebrain and hippocampus, and it may even prevent or delay the positive effects of running on hippocampal neurogenesis (Barrientos et al., 2003; Leasure and Decker, 2009; Scaccianoce et al., 2006; Stranahan et al., 2006). In fact, social isolation is thought to be a major source of stress, and animals living in social isolation tend to show higher level of glucocorticoids and stress hormones (Serra et al., 2005; Toth et al., 2011; Weiss et al., 2004). Stress-induced glucocorticoid level has also been shown to decrease neuronal progenitors proliferation in the hippocampus (Gould and Tanapat, 1999). Interestingly, when

we compared the two control groups in our study, the singly-housed mice lived in complete social isolation and the group-housed mice exposed to a dynamic social interaction, neither groups showed any significant difference in the level of BDNF. One could also argue that the use of male mice in our EE paradigm can potentially create equal amount of stress and tension when compared to those that were housed in single standard housing. Paradoxically, few reports showed that the upregulation of neurogenesis by EE or physical exercise is in fact associated with higher level of corticosterone (Moncek et al., 2004; van Praag et al., 1999b). Apparently, the positive effect of EE is so strong that it somehow overrides the negative effect of stress and/or increased level of stress hormones. Alternatively, this data indicates that social interaction may not be the key component in EE-induced BDNF level in the brain.

Additionally, we have shown that the mRNA level of IGF-1 is distinctively increased in APPswe/PS1ΔE9 and not in the nontransgenic mice following EE. IGF-1 has been implicated in promoting cell survival signaling pathways following EE, and it has been shown to increase neurogenesis through its survival promoting capacity (Aberg et al., 2000; Lichtenwalner et al., 2006). IGF-1 mediates the activation of IGF-1 receptors and activates multiple downstream molecular cascades, including the PI3K/Akt pathway and the c-Src non-receptor tyrosine kinase, thereby modulating cell proliferation and cellular metabolism. IGF-1 receptor activity plays a role in BDNF-mediated effect of physical activity on brain function. It has been shown that IGF-1 signaling via the IGF-1 receptor is necessary for the exercise-induced upregulation of BDNF (Ding et al., 2006). Increased IGF-1 production following exercise training may interact with BDNF to modulate synaptic plasticity, but the nature of the overlap between the exercise-induced regulation of BDNF and IGF-1 is yet to be determined. Altogether, despite of the inconsistency in our findings with IGF-1 in

nontransgenic and APPswe/PS1∆E9 mice, these data provide a valuable information regarding the potential role of EE-induced neurotrophic factors in regulating synaptic functions.

2. <u>Environmental enrichment upregulates gene expression of molecular targets associated with BDNF-dependent signaling pathway(s).</u>

The binding of mature BDNF to TrkB receptors can activate several downstream signaling cascades. TrkB signaling cascades can be further divided into three independent sub-pathways; 1. PI3K/Akt (implicated in cell survival), 2. Ras/Erk (implicated in cell differentiation) and 3. PLC-γ/PKC (implicated in synaptic plasticity) (Ohira and Hayashi, 2009). It is not known which sub-pathway(s) downstream of TrkB activation is directly mediated by EE. Our goal was to investigate these molecular signaling pathway(s) and determined whether they were modified by EE in our AD mouse. To achieve this, we characterized the alterations in gene expression of molecular targets associated with downstream signaling pathways of BDNF upon activation by EE. Specifically, we examined the expression levels of a wide range of potential downstream targets of BDNF signaling cascades, including TrkB receptor, CREB, CBP, Glutamate AMPA receptors, NMDA receptors, CaMKIV and CaMKII.

We started by looking at the gene expression of BDNF receptor, TrkB. Previous studies have suggested that the expression level of TrkB receptor is decreased in the frontal cortex and hippocampal formation in AD, while the truncated form of TrkB receptor is increased in association with decreased BDNF level in these brain regions (Connor et al., 1997; Ferrer et al., 1999). The decrease in BDNF/TrkB expressions may be either a cause or an effect of neuronal degeneration in AD. Our data provides an evidence for the increase of BDNF-TrkB signaling following EE. We showed the upregulation of TrkB gene

transcription in both the nontransgenic and APPswe/PS1ΔE9 mice following EE, suggesting an activation of BDNF/TrkB-dependent signaling pathway (**Figure 18**). To confirm BDNF-mediated TrkB activation following EE, we further assessed the gene transcription of CREB, a well-defined downstream target associated with BDNF-activated pathways. Expression of CREB is critical for the formation of long-term memory and learning (Barco et al., 2003; Sakamoto et al., 2011). Dysregulation of CREB has been implicated in a number of neurodegenerative diseases, including Alzheimer's disease (Ma et al., 2007). In this study, we have shown that CREB mRNA level is dramatically induced following EE in both wild type and transgenic mice, further proved that there is an increased activation of BDNF-TrkB signaling following EE in our AD mice (**Figure 18**).

3. <u>Environmental enrichment alters gene expression of molecular targets associated with BDNF-mediated synaptic plasticity.</u>

BDNF-mediated TrkB activation has been demonstrated to promote neuronal synaptic activity via the activation of the CREB transcription factor, which drives the expression and the activation of intracellular signaling pathways via the action of two types of glutamate-gated ion channels, AMPA and NDMA receptors. BDNF increases GluR1 and GluR2 mRNA and the subunits levels in the hippocampal neurons, and it also increases the mRNA and protein levels for NMDA receptor subunits, NR1, NR2A and NR2B (Caldeira et al., 2007a; Caldeira et al., 2007b). The function of NMDA receptors has been reported to be reduced significantly in AD patients, possibly contributing to memory deficits (Greenamyre et al., 1987). BDNF increases the number of NMDA receptors and thereby upregulates the receptor activity. Moreover, transcription of NMDA receptor is regulated by cAMP signaling pathway, most likely through the binding of CREB via PKA-dependent pathway (Lau et al., 2004). Assuming that the activation of glutamate receptors is required for the induction of

LTP and synaptic plasticity, we would anticipate to see the upregulation of glutamate receptors. Indeed, our data showed that the mRNA expression level of NMDA1 receptor is significantly induced following EE in both wild type and transgenic mice, however, we did not observe any significant changes in GluR1 expression level (**Figure 19**). Our finding is consistent with a previous report that showed EE-induced upregulation of the regional-specific NMDA mRNA in the hippocampus. However GluR1 mRNA level in the hippocampus is unchanged following EE (Andin et al., 2007).

To further elucidate the molecular signaling pathways of EE-induced BDNF increase and its roles in regulating synaptic plasticity, we further examined the expression of molecular targets downstream of NMDA and AMPA-activated signaling pathways. Two of the crucial targets studied were CaMKIV and CaMKII. As mentioned earlier, CaMKIV is an important Ca^{2+}-activated CREB kinase *in vivo*, and it can also act as a kinase for the CREB binding protein (CBP). The activation via AMPA receptors and the autophosphorylation of CaMKII have been shown to be critical for LTP formation (Fukunaga et al., 1993). Conversely, analysis of gene expression by RT-PCR did not show any significant difference in mRNA levels of CaMKIV or CaMKII following EE. Previous research studies reported the increased mRNA and protein expression of synaptic proteins, such as synaptophysin and PSD-95 in many major brain regions, such as forebrains, hippocampus, hypothalamus following EE and classic conditioning training (Nithianantharajah et al., 2004; Rampon et al., 2000; Skibinska et al., 2001). However, in our studies we did not observe any significant changes in the PSD-95 mRNA expression level in the hippocampus following EE (**Figure 19**).

Gene expression of BDNF is controlled by multiple activity-dependent and tissue-specific promoters. Four BDNF promoters have been previously identified in rat, each driving the transcription of BDNF mRNAs containing one of the four 5' non-coding exons

spliced to the common 3'coding exon. In AD, studies have shown the downregulation of specific transcripts of BDNF in the cortical region of the human and mouse brains (Aid et al., 2007; Garzon et al., 2002; Garzon and Fahnestock, 2007; Peng et al., 2009). Therefore it is critical to study how BDNF gene expression is regulated and processed, especially in our transgenic mice. Future experiments will examine the expression of different BDNF mRNA transcripts in APPswe/PS1ΔE9 mice following EE, and how they might affect the function and regulation of BDNF and its signaling pathways in AD. It is also not known whether EE-induced BDNF expression is due to the increased production of BDNF or the increased processing of immature proBDNF into mature BDNF. Exercise has been shown to increase the level of mature BDNF and proBDNF proteolytic cleavage-related genes (Ding et al., 2011; Sartori et al., 2011). This leads to the examination of the mechanism by which EE affects the metabolism of BDNF and its related molecular targets, such as tissue-type plasminogen activator (tPA). Interestingly, the cleavage of proBDNF by tPA has been shown to be essential for long-term hippocampal plasticity (Pang et al., 2004). Here our data showed a significant upregulation of tPA mRNA expression following EE, suggesting that the proteolytic processing of proBDNF into mature BDNF may be enhanced following EE.

While mRNA expression level does not always reflect the expression at the protein level, it is an important tool for the preliminary screening for a broad spectrum of molecular targets. This allows us to analyze a wider range of molecular targets and narrow down the important ones. Understanding which genes are affected by EE will enable us to further elucidate the signaling pathways by which EE promotes plasticity and ameliorate AD pathology. It is, however, important that the expression of genes identified as altered by real-time PCR, will be validated on the protein levels.

4. <u>Environmental enrichment alters expression level of kinases implicated in AD pathology.</u>

Another interesting aspect of EE-induced BDNF signaling involves its potential role in attenuating the process of tau hyperphosphorylation. The enhanced levels of BDNF are specifically thought to underlie tau dephosphorylation in the neurons (Elliott et al., 2005). In this study, we aimed at examining the regulation of tau phosphorylation by environmental enrichment. In particularly, we were interested in studying how EE modulates kinase activities involved in tau phosphorylation. Several protein kinases have been implicated in the pathological process of neurofibrillary tangle formations (Ferrer et al., 2005). In Alzheimer's disease, GSK3β and CDK5 have been studied extensively as the key kinases that phosphorylate tau *in vitro* and *in vivo* (Cruz and Tsai, 2004; Frame et al., 2001; Hernandez et al., 2009; Noble et al., 2003). Previous reports showed an increase in the levels of the active form of GSK3β in the AD brains, suggesting that overactivation of GSK3β activity may result in an abnormal tau phosphorylation (Baum et al., 1996; Kaytor and Orr, 2002). Other studies suggest that GSK3β can phosphorylate kinesin light chains and negatively regulate kinesin-based motility (Morfini et al., 2002b).

Other kinases, such as casein kinase 2 (CK2), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase have also been implicated for their roles in tau hyperphosphorylation in AD (Matsuda et al., 2001; Reynolds et al., 1997). CK2 is an ubiquitously-expressed serine/threonin protein kinase with multifunctional roles in signal transduction, cell-cell adhesion, cytoskeleton structure and tumorigenesis (Meggio and Pinna, 2003). Like GSK3β and Cdk5, CK2 can phosphorylate tau, APP and kinesin-1 (Avila et al., 1994; Hanger et al., 2007; Schafer et al., 2008; Walter et al., 2000), and the upregulation of CK2 can resemble similar effects to those observed by oligomeric Aβ in

disrupting axonal transport (Pigino et al., 2009). Having more than 300 potential substrates involved in the signal transduction, the physiological role of CK2, especially in the context of Alzheimer's disease, is still poorly understood. CK2, which is present in high concentrations in the hippocampus, is also rapidly activated in the CA1 region during the induction of LTP, suggesting an important role for CK2 in hippocampal LTP formation and in synaptic plasticity (Blanquet, 2000; Charriaut-Marlangue et al., 1991). However, a different research group reported that the activation of CK2 may impair spatial memory formation via crosstalk with PI3K/Akt dependent signaling pathway and through the inactivation of glucocorticoid-inducible kinase 1 (SGK1)(Chao et al., 2007). In addition, CK2 is also found to facilitate the neurotrophic response of BDNF and Glial cell-derived neurotrophic factor (GDNF) in the hippocampus and in dopaminergic neurons, respectively, via MEK/ERK signaling cascades. (Blanquet, 1999).

Interestingly, we have shown a significant reduction in levels of hyperphosphorylated tau using PHF-1 antibody and the increased expression of kinesin-1 in the brains of AD mice following EE. Taken together, this may suggest that experience in an enriched environment downregulates activity of kinase(s) that involve in phosphorylation of tau and kinesin-1. Based on our previous observation that experience in EE attenuates PHF-1 immunoreactivity and enhances kinesin-1 expression, we examined the expression level of GSK3β kinase, as well as other kinases that participate in tau pathogenesis in the brains of APPswe/PS1ΔE9 mice following EE. In this study, we showed that the protein expression level of active-form of GSK3β is significantly downregulated in nontransgenic, and not in the APPswe/PS1ΔE9 mice following EE. This provides an indirect evidence that the activity of GSK3β kinase can be modulated by EE (**Figure 23, 24**).

Similarly to GSK3β, we were able to detect an increase of Akt activation in the nontransgenic, and not in the APPswe/PS1ΔE9 mice (**Figure 25, 26**). Nevertheless, our

results support previous studies suggesting that EE and/or physical exercise activates PI3K pathway and regulates Akt and GSK3β activities in human skeletal muscle of healthy subjects (Chen and Russo-Neustadt, 2005; Sakamoto et al., 2004). This may suggest that in nontransgenic mice, EE may operate via PI3K/Akt signaling pathway.

Next, using specific CKIIα antibodies, we have shown that the expression level of CK2 was significantly lower in the nontransgenic mice following EE (**Figure 27**). Protein kinase CK2 is thought to be a constitutively active kinase, therefore the expression level shown by Western blot may directly reflect the kinase activity of CK2 (Litchfield, 2003). However, when we compared the nontransgenic and APPswe/PS1ΔE9 mice maintained in standard housing condition, we did not observe any significant difference in CK2 activity. Finally, we examined the expression level of Cdk5, in the brains of EE versus SH mice. While the expression of full-length Cdk5 did not show any significant change (data not shown), additional analyses of its activators, p25 or p35 are essential to further characterize potential changes in Cdk5 kinase activity.

This part of the work provides three important observations: (1) We have identified BDNF-mediated signaling pathways modulated by EE in nontransgenic mice, i.e. Akt and GSK3β. (2) We have identified impaired modulation of Akt and GSK3β signaling pathway by EE in FAD-linked APPswe/PS1ΔE9 mice. (3) We have determined a connection between EE and the upregulation of CREB, a signaling molecule critical for learning and for the formation of long-term memory. One possibility is that the expression of human APP and PS1 mutants may alter cellular signaling pathways in APPswe/PS1ΔE9 mice, compared to the nontransgenic mice. Evidently, GSK3β activity is altered in AD patients and in many AD mouse models (Hernandez et al., 2009; Hernandez et al., 2010; Takashima et al., 1998). While we did not observe any changes in GSK3β expression level in 2-month old

APPswe/PS1ΔE9 mice, compared to their age-matched littermate controls (**Figure 21**), it is very likely that the effect of GSK3β on AD pathology in transgenic mice is more apparent at an older age. This is supported by our observation that the expression level of the active-form of GSK3β is increased with age (**Figure 20**).

GSK3β is an ubiquitously expressed kinase, and unlike most kinases, it has a relatively high activity in resting, unstimulated cells. Its activity is normally reduced in response to a variety of extracellular stimuli that typically induce a *rapid* and *reversible* decrease in enzymatic activity. Thus, it is possible that only specific pools of GSK3β are differentially modulated by AD and/or EE. Currently, it is not known which fraction of GSK3β is regulated by EE. It is also not known whether mutants APP and PS1 have any effects on the distribution of GSK3β or its metabolism in the cell, which could affect the expression and activity of GSK3β analyzed. Previous study has shown that mutant mice with GSK3 mutations that prevent phosphorylation of GSK3 can survive into adulthood without any obvious phenotype in their CNS. This provides an unequivocal genetic evidence that phosphorylation of GSK3 at their N-terminal serine residues is not the only mechanism that regulates GSK3 activity in the CNS (McManus et al., 2005). More importantly, it is becoming increasingly apparent that changes in GSK3 activity are not always accompanied by changes in their phosphorylation status. More information concerning the cellular regulation of GSK3 activity would aid in understanding of EE-induced modulation of this enzyme.

In summary, our data supports our hypothesis, at least in part, that EE can modulate activity of several kinases, some of which may promote PI3K/Akt neuronal survival pathway in the nontransgenic mice. Equally important, our results have demonstrated that while experience in an EE leads to a significant deactivation of kinase(s) associated with AD, experience of APPswe/PS1ΔE9 mice in an enriched environment fails to exert the same

effect. This suggests that the effects of mutants APP and PS1 dominate the effect of EE on kinase activities that are successfully modulated in wild type animals. Alternatively, we cannot exclude the possibility that the signaling pathways modulated by EE are different in APPswe/PS1ΔE9 and in nontransgenic mice. In fact, it will be very interesting to look at the effect of EE on sporadic AD mouse model, such as ApoE mutant mice and determine whether the effects of EE are similar to those that were observed in APPswe/PS1ΔE9 and in nontransgenic mice.

Altogether, the latter parts of this thesis work have provided the evidence for several mechanistic explanations for the effect of EE on modulating Alzheimer's disease pathology, neuroplasticity and synaptic functions. Based on the evidence of our studies, we reasoned that the experience in an enriched environment results in a higher level of neurotrophic factors (i.e. BDNF and NGF) in the hippocampus, which in turn facilitates the downstream molecular signaling pathways, leading to the effects that we observed in the study (**Figure 28**).
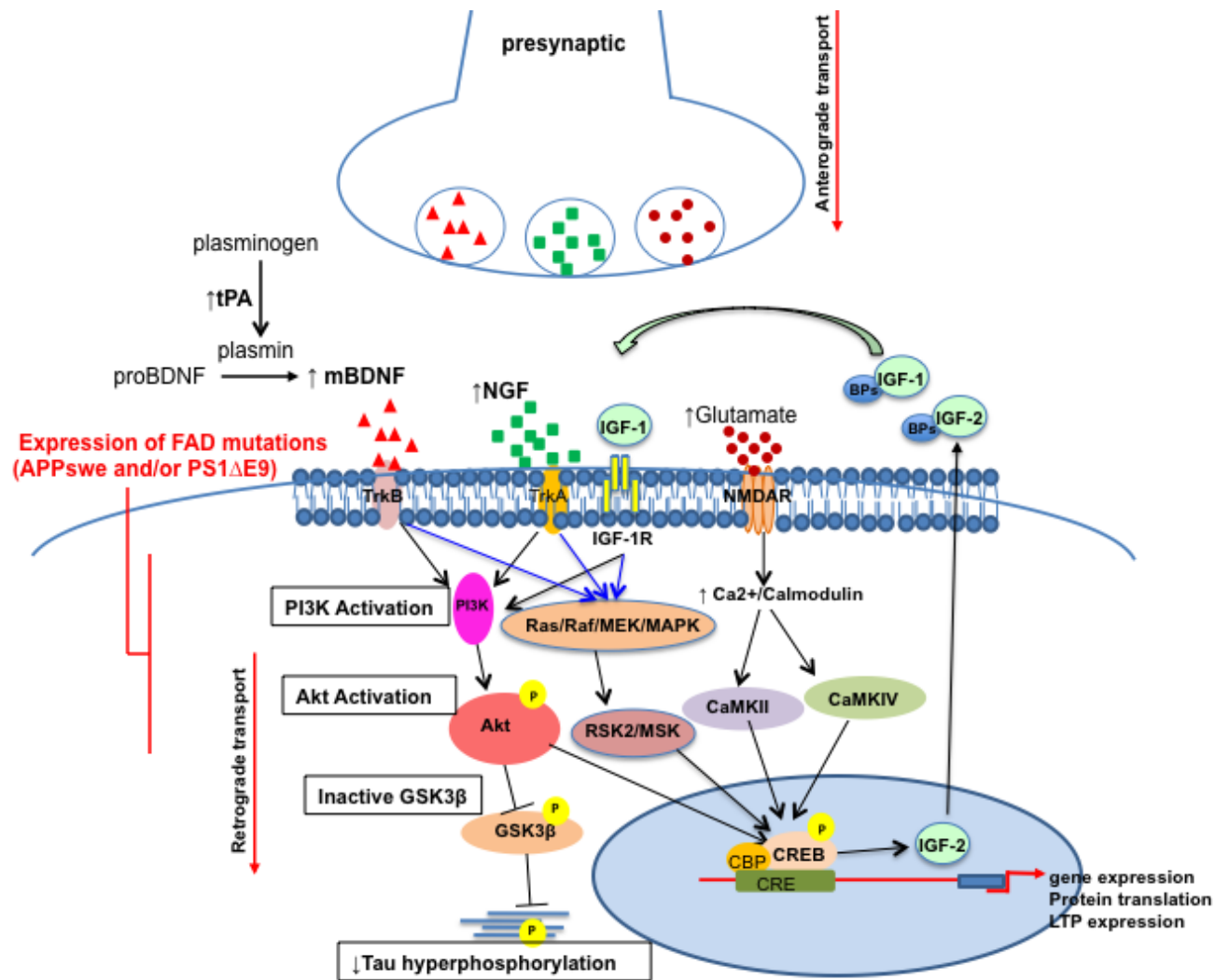
FIGURE 28 The cross talk between EE-mediated signaling pathways.

A proposed molecular network demonstrating the interaction between different possible signaling pathways mediated by EE-induced neurotrophic levels in the hippocampus. Many of these signaling pathways have been shown in our study to directly modulate synaptic function and kinase activities.

VI. CONCLUSION AND FUTURE DIRECTIONS

This thesis has provided numerous important pieces of evidence to support the critical roles of EE in modulating Alzheimer's disease pathology and neuroplasticity. Numerous studies have suggested that the extent of adult neurogenesis declines with age, suggesting the possibility that reduction in neurogenesis may account for, at least in part, cognitive deterioration in the elderly (Kempermann et al., 2004; Seki and Arai, 1995). This raises the intriguing question of the fate of neurogenesis in the disease progression of AD. Unfortunately, information regarding neurogenesis in AD patients and in the transgenic animal models is very limited, and the existing data is inconclusive. In the first part of this work, we have examined several aspects of postnatal neurogenesis in FAD-linked APPswe/PS1ΔE9 mice, and determined that cell proliferation and cell differentiation of NPCs is severely impaired at a very early age, long before the onset of amyloid deposition or cognitive deficits. In addition we showed that tau hyperphosphorylation takes place early in NPCs and in the neurogenic regions and may contribute to the progression of the disease and impaired neurogenesis, which, in turn, may compromise learning and memory.

Attenuation or prevention of cognitive deterioration and neurodegeneration are top priority when it comes to treating AD. The ability to modulate neurogenesis may provide a potential therapeutic approach to slow down or even prevent memory loss and cognitive impairment. The ability to induce formation of new neurons and support their functional integration in local circuits may compensate for loss of degenerating neurons and memory impairments. This work has demonstrated the role of EE in regulating neurogenesis in the adult brain of FAD mouse model by identifying alterations in cell signalings and critical players in neurogenesis and AD. This is also very crucial for the development of neural stem cell- based therapeutic approaches.

In this work, we showed that rescue of impaired neurogenesis in APPswe/PS1ΔE9 mice following EE was accompanied by the amelioration of amyloid and tau pathology and enhancement of synaptic plasticity. The novelty of this part of the study is that it is the first study to examine the effect of EE on precursors of AD pathological hallmarks, namely the soluble oligomeric Aβ peptides and hyperphosphorylated tau levels in FAD mouse model. This suggests that early behavioral intervention is a valid and successful approach for the amelioration of neuropathology. In addition, we are the first to demonstrate the effect of EE on LTP and synaptic function in the APPswe/PS1ΔE9 mouse model. The impacts of EE on neurodegenerative disease and human disorders are beyond the scope of this work. In the future, discovering a way to translate the application of EE into human clinical trials will benefit tremendously the development of non-drug therapeutic intervention for AD.

The ultimate goal of this work is to unravel the molecular signaling pathways underlying the effects of EE on AD pathology and neuroplasticity. We have presented novel information suggesting that EE regulates (1) several neurotrophin pathways, such as BDNF and its downstream signals, implicated in learning and memory and synaptic plasticity, (2) kinases implicated in AD-linked tau phosphorylation and axonal transport motors, crucial for neuronal viability.

Intriguingly, this study also opens up some important questions that should be addressed in future experiments, Two of these questions are outlined below: (1) what component(s) of the EE is responsible for the effect on disease pathology and neuroplasticity? One important future experiment is to dissect out different components of EE and evaluate each of the effects individually. (2) Interestingly, our study suggests that a particular signaling pathway, i.e. PI3K/Akt/GSK3β pathway, is impaired in our APPswe/PS1ΔE9 mouse model and cannot be rescued by EE. To further elucidate the

molecular mechanisms underlie impaired PI3K/Akt/GSK3β pathway in APPswe/PS1ΔE9 mice, we are eager to examine the effect of FAD mutations on these signaling pathways. Understanding the effects of FAD mutants on cellular processes will not only provide mechanistic explanations for disease formation and progression, it will also provide further insights into the specificity of the effect of EE on AD.

In conclusion, this work has shown that despite severe pathology, EE can effectively downregulate AD pathology and enhance neuroplasticity in the brains of FAD-linked APPswe/PS1ΔE9 mice. The knowledge of this work has provided a very extensive and valuable information concerning a mechanistic link between EE, adult neurogenesis and FAD, and it may provide critical information for the development of therapeutic intervention for AD.

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ANOVA Table 1: Two-way analysis of variance for total BrdU+ cell counts

Effect	Df	Sum of squares	Mean square	F value	Pr > F
Housing condition	1	48815190	48815190	54.88182	*< 0.0001*
Genotype	1	9643799	9643799	10.84231	*0.0046*
Interaction	1	76865.24	76865.24	0.0864179	0.7726
Residual (Error)	16	14231360	889460.1		
Total	19	72767214.24			

ANOVA Table 2: Two-way analysis of variance for BrdU+/DCX+ cell counts

Effect	Df	Sum of squares	Mean square	F value	Pr > F
Housing condition	1	8471451	8471451	19.21979	*0.0005*
Genotype	1	400679.9	400679.9	0.9090515	0.3545
Interaction	1	291053.8	291053.8	0.6603346	0.4284
Residual (Error)	16	7052273	440767.1		
Total	19	16215457.7			

ANOVA Table 3: Two-way analysis of variance for BrdU+/GFAP+ cell counts

Effect	Df	Sum of squares	Mean square	F value	Pr > F
Housing condition	1	231101	231101	22.43	*0.0002*
Genotype	1	16987	16987	1.648	0.2175
Interaction	1	3962	3962	0.3845	0.5439
Residual (Error)	16	164880	10305		
Total	19	416930			

ANOVA Table 4: Two-way analysis of variance for BrdU+/S100β+ cell counts

Effect	Df	Sum of squares	Mean square	F value	Pr > F
Housing condition	1	3122	3122	1.85	0.1926
Genotype	1	1594	1594	0.9448	0.3455
Interaction	1	1846	1846	1.094	0.3111
Residual (Error)	16	26991	1687		
Total	19	33553			

ANOVA Table 5: Two-way analysis of variance for BrdU+/NeuN+ cell counts

Effect	Df	Sum of squares	Mean square	F value	Pr > F
Housing condition	1	117539	117539	18.09	***0.0011***
Genotype	1	7749	7749	1.193	0.2962
Interaction	1	1227	1227	0.1889	0.6716
Residual (Error)	12	77968	6497		
Total	15	204483			

APPENDIX II

ANOVA Table 1: One-way analysis of variance for hippocampal BDNF ELISA

Effect	Df	Sum of squares	Mean square	F value	Pr > F
Treatment	5	788.4	157.7	24.78	*<0.0001*
Residual (Error)	28	178.4	6.362		
Total	33	966.5			

ANOVA Table 2: One-way analysis of variance for cortical BDNF ELISA

Effect	Df	Sum of squares	Mean square	F value	Pr > F
Treatment	5	16.24	3.248	1.067	*0.3990*
Residual (Error)	29	88.30	3.045		
Total	34	104.5			

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University of Illinois at Chicago, USA 2012
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First Honor Undergraduate Independent Study Award 2006
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Undergraduate Level Research Scholarship 2005
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Academic Excellence Scholarship 2004, 2005
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Hu, Y.-S., Xu, P., Pigino, G., Brady, S.T., Larson, J., & Lazarov, O. (2010). Complex environment experience rescues impaired neurogenesis, enhances synaptic plasticity, and attenuate neuropathology in Familial Alzheimer's Diseases-linked APPswe/PS1ΔE9 mice. *FASEB J.* 24(6): 1667-81.

Demars, M*., **Hu, Y.-S.***, Gadadhar, A., & Lazarov O. (2010). Neurogenesis is impaired early in life in an animal model of Familial Alzheimer's disease. J *Neurosci Res.* 88(10): 2103-17.
Contribute equally in this study

Perez, S.E., Berg, B.M., Moore, K.A., He, B., Counts, S.E., Fritz, J.J., **Hu, Y.-S.**, Lazarov, O., Lah, J.J., & Mufson, E.J. (2009). DHA diet reduces AD pathology in young APPswe/PS1 Delta E9 transgenic mice: possible gender effects. *Journal of Neurosci Res*, 88(5): 1026-40.

Newsletter Article

Hu, Y.-S. (2005) Reaching out to the community- Pharmacists as the catalysts of changes. National Taiwan University, College of Medicine Newsletter. Volume 150, page 7-8. (Publication in Mandarin)

SCIENTIFIC PRESENTATIONS

Conference Presentations

Hu, Y.-S. and Lazarov, O. (2011) Molecular signaling mediating the effect of environmental enrichment on Alzheimer's disease neuropathology. Nanosymposium oral presentation given at the 41th annual meeting of the Society of Neuroscience, Washington D.C., November 12-16.

Lazarov, O., **Hu, Y.-S.**, Xu, P., Pigino, G., Brady, S.T., Larson, J. (2011). *Environmental enrichment as a key modulator of brain plasticity in Alzheimer's disease.* Poster presented at the 11th International Conference On Alzheimer's & Parkinson's diseases, Barcelona, Spain, March 9-13

Hu, Y.-S., Xu, P., Pigino, G., Brady, S.T., Larson, J., & Lazarov, O. (2010). †*Environmental enrichment as a key modulator of brain plasticity and neuropathology in Alzheimer's disease.* Nanosymposium oral presentation given at the 40th annual meeting of the Society of Neuroscience, San Diego, CA, November 13-17.
† Has also been selected as Hot Topics for Neuroscience 2010, lay language summary is published for media press in the Hot Topics Book, available at the annual meeting.

Hu, Y.-S., Xu, P., Pigino, G., Brady, S.T., Larson, J., & Lazarov, O. (2010). *Rescue of impaired neurogenesis and attenuation of neuropathology in FAD-linked transgenic mice by environmental factors.* Poster presented at the annual meeting of Alzheimer's Association International Conference on Alzheimer's disease (ICAD), Honolulu, July 10-15.

Hu, Y.-S., Xu, Larson, J., & Lazarov, O. (2009). *Enrichment-induced plasticity rescues impaired neurogenesis and neuronal phenotype in Alzheimer's disease.* Poster presented at the annual meeting of Society of Neuroscience, Chicago, October 17-21.

Perez, S.E., Berg, B.M., Moore, K.A., He, B., Counts, S.E., Fritz, J.J., **Hu, Y.-S.**, Lazarov, O., Lah, J.J., & Mufson, E.J. (2009) *Gender affects docosahexaenoic acid (DHA)*

reduction in beta-amyloid and synaptic protein levels in the forebrain of young APPswe/PS1 Delta E9 transgenic mice. Poster presented at the annual meeting of Society of Neuroscience, Chicago, October 17-21.

Hu, Y.-S., & Lazarov, O. (2009). *Modulation of brain plasticity in Alzheimer's disease by environmental factors.* Poster presented at the annual meeting of American Association for the Advancement of Science (AAAS), Chicago, February 12-16.

Demars, M., Gadadhar, A., **Hu, Y.-S.**, & Lazarov O. (2008) *Familial Alzheimer's Disease pathology differentially affects discrete neurogenic niches in the adult brain.* Poster presented at the annual meeting of Alzheimer's Association International Conference on Alzheimer's disease (ICAD), Chicago, July 26-31.

Chiu, L., Ruan, C.-T., Liu, P.-H., **Hu, Y.-S.**, & Kung, F.-L. (2005). *The intercellular domain of amyloid precursor protein interacts with flotillin-1, a lipid raft protein.* Poster presented at the annual meeting of Chinese Medicinal Chemistry Symposium, Taiwan.

Invited Talks

Hu, Y.-S. (2012) *Modulation of Alzheimer's disease pathology and neuroplasticity by environmental factors.* National Institute on Aging, Laboratory of Neuroscience, Baltimore, MD, April 6.

Hu, Y.-S. (2009) *Neuropathology, plasticity and learning in Alzheimer's mouse models: Effects of enriched experience.* Illinois Department of public health, Alzheimer's disease advisory committee annual meeting, Rush University, Chicago, December 10.

WORK EXPERIENCE

Teaching Assistant
- Department of Anatomy and Cell biology, University of Illinois at Chicago, 2009.
- Community College Pharmacy Education Program, Ban Chiao, Taiwan, 2003-2004

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Year, month, volume, issue, pages, and DOI:

2010, June, volume 24, issue no. 6, pages 1667-1681, DOI: 10.1096/fj.09-136945

Names of all authors: Yuan-Shih Hu, Peng Xu, Gustavo Pigino, Scott T. Brady, John Larson, Orly Lazarov

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