Cell. Mol. Life Sci. 65 (2008) 2372 – 2384
1420-682X/08/152372-13
DOI 10.1007/s00018-008-8053-y

Cellular and Molecular Life Sciences

Review

Behavior of neural stem cells in the Alzheimer brain

B. Waldau and A. K. Shetty*

Department of Surgery (Neurosurgery), Duke University Medical Center, Durham, NC 27710 (USA) and Medical Research and Surgery Services, Veterans Affairs Medical Center, Durham, NC 27705 (USA), Fax: +9 192 864 662, e-mail: Ashok.Shetty@Duke.Edu

Received 29 January 2008; received after revision 16 March 2008; accepted 18 March 2008
Online First 26 May 2008

Abstract. Alzheimer's disease (AD) is characterized by the deposition of β-amyloid peptides (Aβ) and a progressive loss of neurons leading to dementia. Because hippocampal neurogenesis is linked to functions such as learning, memory and mood, there has been great interest in examining the effects of AD on hippocampal neurogenesis. This article reviews the pertinent studies and tries to unite them in one possible disease model. Early in the disease, oligomeric Aβ may transiently promote the generation of immature neurons from neural stem cells (NSCs). However, reduced concentrations of multiple neurotrophic factors and higher levels of fibroblast growth factor-2 seem to induce a developmental arrest of newly generated neurons. Furthermore, fibrillary Aβ and down-regulation of oligodendrocyte-lineage transcription factor-2 (OLIG2) may cause the death of these nonfunctional neurons. Therefore, altering the brain microenvironment for fostering apt maturation of graft-derived neurons may be critical for improving the efficacy of NSC transplantation therapy for AD.

Keywords. Alzheimer's disease, Alzheimer's pathology, dentate neurogenesis, neural progenitor, neural stem cell, stem cell renewal, stem cell differentiation, stem cell graft.

Introduction

Alzheimer's disease (AD) afflicts ~15 million people worldwide [1]. In the United States of America, more than 4 million people are suffering from AD, and their numbers are predicted to triple by 2050 [2]. The clinical symptoms of AD are exemplified by progressive deterioration of cognitive function, psychiatric problems and dementia [3]. Thus, persons afflicted with AD steadily lose their memory and ability to learn, reason, make judgments, communicate and carry out daily activities.

Persistence of neurogenesis in the dentate gyrus of the hippocampal formation during adulthood was identified more than a decade ago [4]. Adult neurogenesis is a process by which new neurons are produced from neural stem/progenitor cells (NSCs) in the adult brain. Neural stem cells are the self-renewing, multipotent cells that are capable of unlimited proliferation and generating the central nervous system (CNS) phenotypes such as neurons, astrocytes and oligodendrocytes [5]. On the other hand, progenitor cells are either multipotent or bipotent, have limited proliferation potential and lack self-renewal ability. Because of the difficulty in distinguishing NSCs from neural progenitors *in vivo*, classifying them as neural stem/progenitor cells (NSCs) has been the norm in most of the *in vivo* studies.

Adult neurogenesis occurs throughout life in two 'neurogenic regions' of the brain: (i) the subventricular zone (SVZ) lining the lateral ventricles of the

* Corresponding author.

brain and (ii) the subgranular zone (SGZ) of the dentate gyrus in the hippocampal formation [6, 7]. In the rodent brain, proliferation of NSCs in the SVZ produces a large pool of neuroblasts which migrate into the olfactory bulb through a pathway called the rostral migratory stream. Once they reach the olfactory bulb, they differentiate into olfactory interneurons. On the other hand, proliferation of NSCs in the SGZ results in production of new granule cells and glia, which migrate mainly into the dentate granule cell layer (GCL, Fig. 1) In the GCL, they mature, send dendrites into the molecular layer and axons into the stratum lucidum of the CA3 via the dentate hilus where they make synaptic contacts with CA3 pyramidal neurons.

Many life conditions can modulate the rate of hippocampal neurogenesis. The positive regulators of neurogenesis in the young brain include physical exercise [8, 9] and environmental enrichment [10]. However, brain injury inflicted through acute seizures or cerebral ischemia also triggers increased neurogenesis in the young adult brain [11, 12] but not in the aged brain [13]. In addition, neurogenesis is sensitive to the concentrations of a variety of neurotrophic factors and molecules [14]. The negative regulators include stress [15], aging [16–19] and chronic neurodegenerative conditions such as temporal lobe epilepsy [20]. Hippocampal neurogenesis is considered important for functions such as learning, memory and mood, demonstrated by the increased survival of newborn neurons in the hippocampus through hippocampal-dependent learning and memory tasks [21–23] and through increased neurogenesis observed after antidepressant therapy [24].

The goals of this review are to discuss the extent of changes in dentate neurogenesis during AD, the role of AD pathology in altering the behavior of NSCs and their differentiation in the hippocampus, and the potential strategies for improving neurogenesis in AD brain.

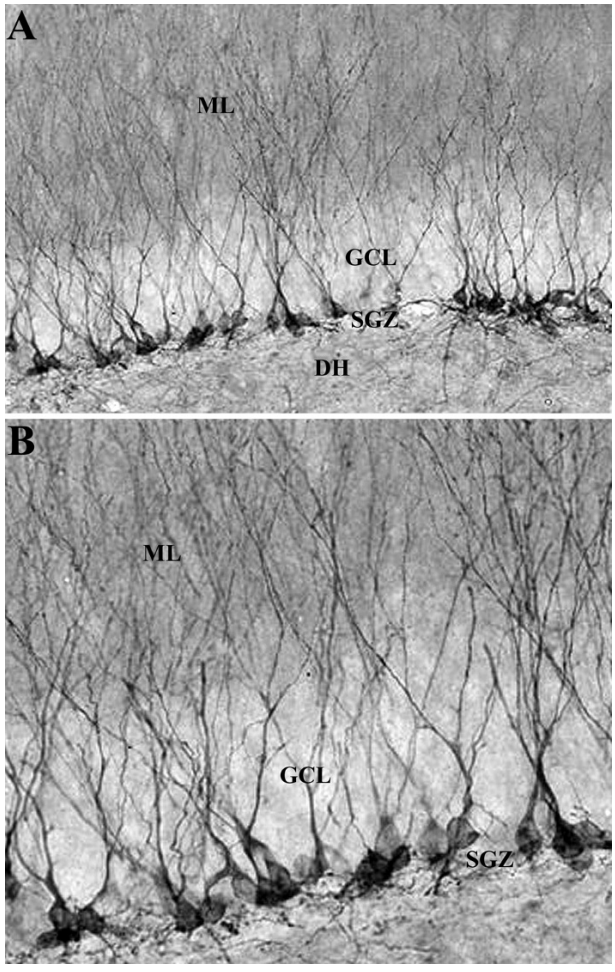

Figure 1. Doublecortin immunostaining of the dentate gyrus from a normal adult rat brain showing newly born neurons (*A*) (magnified view of a region from *A* highlights the morphology of newly born neurons, *B*). Cell bodies of newly born neurons are located in the subgranular zone (SGZ) whereas their dendrites extend through the granular cell layer (GCL) into the molecular layer (ML) These doublecortin-positive cells migrate mainly into the GCL where they mature into full-fledged granule cells. Granule cells send axons into the stratum lucidum of the CA3 via the dentate hilus (DH) where they make synaptic contacts with the CA3 pyramidal neurons.

Basic pathology

The hallmark of AD is the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles [25, 26]. Typically, these plaques are formed in the brain many years before the onset of clinical signs of AD. The primary proteinaceous component of the senile plaques is Aβ [27]. Aβ is produced by cleavage of the amyloid precursor protein (APP) at the N- and C-terminal end by β- and γ-secretase, respectively. Two Aβ proteins are produced by γ-secretase that are 40 or 42 amino acids long and termed $A\beta_{40}$ and $A\beta_{42}$, respectively [28]. The carboxy-terminal length of the Aβ molecule was shown to be critical for determining the formation rate of Aβ fibrils: $A\beta_{42}$ precipitates and forms fibrils much more easily than $A\beta_{40}$ [29]. Typically, $A\beta_{42}$ constitutes only 10% of the total secreted Aβ [30], but *in vitro* experiments have shown that its fraction may be significantly increased in AD [31]. There is also evidence in mouse models that $A\beta_{40}$ inhibits amyloid deposition [32]. Thus, $A\beta_{42}$ has predominantly been used by investigators to examine the effects of Aβ on neurogenesis.

Familial Alzheimer disease

Several genetic mutations have been suggested to contribute to early onset AD usually around 50 years of age. Three genes have been implicated in the development of familial AD (FAD): βAPP, presenilin-1 (PS-1) and presenilin-2 (PS-2). Mutation in βAPP can occur at position 717 (Indiana mutation) substituting valine for isoleucine, phenylalanine or glycine [33–35]. Mutations can also occur at positions 670/671. This mutation was first discovered in a Swedish family with early AD and is referred to as the Swedish double-mutation [36]. Mutations in APP in FAD are close to the sites of cleavage of APP for β- and γ-secretases which may promote the formation of Aβ. The Swedish double-mutation, for example, enhances the activity of β-secretase [31, 37, 38], and the 717 mutation increases fractional $A\beta_{42}$ expression [39]. PS-1 and PS-2 are an integral part of the γ-secretase complex [40, 41], and mutations in these genes have also been shown to cause an increase in $A\beta_{42}$ production [42–44].

Animal models of AD

As neurogenesis in AD has been studied in animal prototypes, it is important to understand the underlying pathology in these models. Transfection of human APP is necessary to cause Aβ deposition in mouse models; however, increased concentrations of mouse APP do not have any effects [45]. To study neurogenesis, human APP (hAPP) has been used as a transgene with either the Swedish double-mutation or mutations at position 717. Expression of hAPP is usually driven in animal models by neuron-specific promoters such as PDGF, Thy-1 or Thy-1.2 or non-specific promoters such as hamster PrP [46]. The PDAPP transgenic line bears the mutation at position 717 and is driven by the neuron-specific PDGF promoter [47]. At the age of 6 months, these animals start to develop deposits of Aβ peptide in the hippocampus [48]. Transgenic mice over-expressing the Swedish double-mutation (APP_{swe}) develop late-onset amyloid pathology [49].

Transgenic animals with missense mutations of PS-1 show accelerated amyloid pathology in the brain by the preferential production of $A\beta_{42}$ [44] while their cognitive impairment is modest [50]. Doubly transgenic mice (APPPS1 mice) with the Swedish double-mutation in APP and a point mutation in PS-1 at position 166 may show cerebral amyloidosis as early as 6–8 weeks post-partum [51]. Deletion of exon 9 in PS-1 co-expressed with the Swedish double-mutation leads to Aβ deposits at the age of 4–5 months [52, 53].

A triple-transgenic mouse model harboring PS1, APP and tau gene mutations has also been developed in order to more closely mimic the AD process [54]. These mice develop both amyloid plaques and neurofibrillary tangles. However, studies examining neurogenesis with this model have not yet been published.

Aggregation states of $A\beta_{42}$

Special attention needs to be paid to the preparation of $A\beta_{42}$ for experiments since it has a strong tendency to self-aggregate. The different aggregation states can have different effects on proliferation and differentiation of NSCs which will be discussed below. $A\beta_{42}$ exists in monomeric, oligomeric and fibrillary form. Monomeric $A\beta_{42}$ can be aggregated to form oligomeric or fibrillary $A\beta_{42}$. An effort has been made to standardize protocols for the preparation of $A\beta_{42}$ in order to minimize lot-to-lot variability that can affect both the aggregation behavior and the effects of $A\beta_{42}$ on neural progenitor cells [55]. The goal of this protocol is to remove the structural history of the preparation, since a small number of pre-aggregated molecules are thought to act as seeds driving further aggregation down a particular pathway. Initially, the lyophilized $A\beta_{42}$ is dissolved in hexafluoroisopropanol, speed-dried and then resuspended in dry DMSO. For the production of oligomers, $A\beta_{42}$ is incubated at 4 °C for 24 h at physiological culture conditions. The production of fibrillary $A\beta_{42}$ is achieved by incubating at 37 °C for 24 h with a low pH and salt concentration.

Which aggregation state is responsible for the pathology?

It is necessary to identify the predominant aggregation state of $A\beta_{42}$ in Alzheimer patients so that *in vitro* experiments can focus on using this aggregation state to study neurogenesis. Looking at samples of post-mortem AD patients, the level of insoluble $A\beta_{42}$ was found to be, on average, 100 times that of control brains, whereas the amount of water-soluble $A\beta_{42}$ was found to be about 6 times that detected in control brains [56]. Any form of $A\beta_{42}$ was considered soluble as long as it had not yet polymerized into its insoluble, filamentous form. Therefore, fibrillary $A\beta_{42}$ constitutes the predominant form of $A\beta_{42}$ detected in deceased AD patients. This may be different in earlier disease stages when $A\beta_{42}$ oligomers have not reached the critical concentration to precipitate into the fibrillary aggregation state.

Another issue of contention is the aggregation state of $A\beta_{42}$ that causes the clinical pathology in AD.

Extraction of $A\beta_{40}$ and $A\beta_{42}$ from post-mortem brain has shown that total levels of these peptides do correlate with the severity of disease progression [57]. Plaque density and total volume of amyloid, however, correlate poorly with the severity of dementia in AD [58, 59]. This observation has led to the hypothesis that $A\beta_{42}$ oligomers are primarily responsible for causing the cognitive decline in AD [60]. This theory is supported by the fact that $A\beta_{42}$ oligomers have been found to be at least as toxic as $A\beta_{42}$ fibrils [61]. Soluble oligomers have also been found to be potent inhibitors of long-term potentiation and might therefore cause the early memory loss seen in AD [62]. A 56-kDa soluble amyloid-β oligomer (Aβ*56) was isolated from an Alzheimer mouse model that showed cognitive impairment before neurodegeneration or amyloid-β pathology. Infusion of this oligomer into young rats reproduced the cognitive impairment seen in the mouse model [63, 64]. Immunization of a transgenic APP mouse line with an antibody that recognizes soluble oligomers led to significant cognitive improvement [65]. Thus, there is significant evidence that $A\beta_{42}$ oligomers play an important role in the clinical pathology of AD.

Effect of AD on neurogenesis in human studies

Post-mortem studies

Post-mortem studies on the status of neurogenesis in human Alzheimer patients have been contradictory. One group of investigators reported an increase in hippocampal neurogenesis in AD after performing Western blot and immunohistochemical analyses on post-mortem tissue [66]. Using markers of immature neuronal cells such as Turned On After Division (TOAD)/Ulip/CRMP-4 (TUC-4), polysialated neural cell adhesion molecule (PSA-NCAM), doublecortin (DCX) and NeuroD, they reported an increased density of such cells in the granule cell layer of Alzheimer brains in comparison to control subjects (Fig. 2) In addition, the overall expression of these marker proteins was found to be increased in Western blot analyses of the hippocampus. The AD brains also exhibited potential new neurons in the CA1 region of the hippocampus. Additionally, the authors reported increased levels of proliferating cell nuclear antigen (PCNA) in the hippocampus of AD brains, suggesting that an increased proliferation of cells likely occurs in the hippocampus of AD brains. However, the possibility of binding of PCNA antibodies to apoptotic cells in the AD brain needs to be ruled out to confirm this finding [67, 68]. Additionally, rigorous quantification of changes in the levels of the above-mentioned proteins will be helpful in future studies for further confirmation of their increase in AD. Based on their data, the authors suggest that AD triggers neurogenesis in an attempt to replace lost neurons, leading to an increased number of immature neurons in the GCL. However, it should be noted that neurogenesis in senile AD cases may also reflect the effects of AD drug therapy. This is because a recent study suggests that administration of AD drugs such as tacrine, galantamine or memantine to mice increases neurogenesis in neurogenic regions of the brain [69].

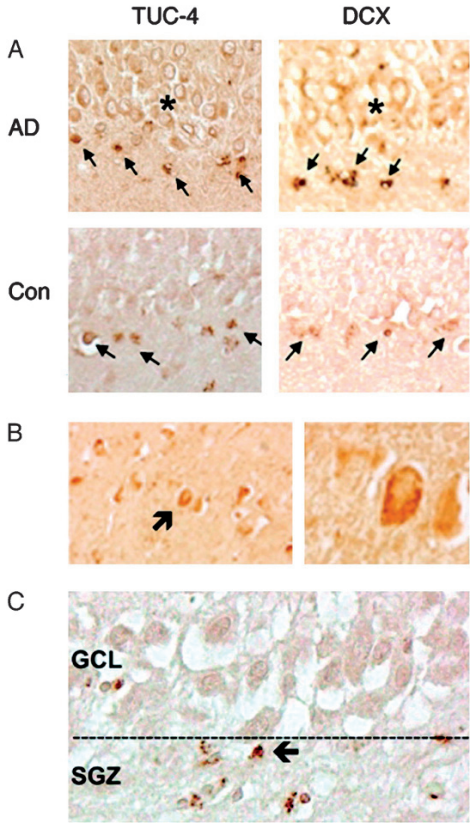

Figure 2. Immunohistochemical evidence for increased production of immature neurons in the dentate gyrus of senile AD patients (Figure reproduced from Jin *et al.*, Proc. Natl. Acad. Sci. USA 101:343–347, 2004). Immunostaining for TUC-4 and doublecortin (DCX) shows the presence of a larger number of immature neurons in the granule cell layer (*) compared to controls (*A*). DCX-positive cells were also found in the CA1 region in this patient population (*B*). Cells in the SGZ undergo apoptosis which is demonstrated by staining for the 10-kDa caspase-8 cleavage product (*C*).

Contrasting with this report are data that found no increased neurogenesis in the presenile Alzheimer hippocampus in post-mortem tissue [70]. Semi-quantitative scoring of DCX showed no difference between Alzheimer patients and normal subjects. Immunohistochemistry for Ki-67, an endogenous marker of cell proliferation that does not undergo post-mortem decay, revealed no change in cell proliferation in the

neuron-rich dentate granule cell layer of AD brains. Although there was an increased density of Ki-67+ cells in CA1 and CA3 regions of the hippocampus, all of these cells appeared to be glia and other non-neuronal cells. Thus, in contrast to the results obtained in senile AD cases, it appears that presenile AD brains do not exhibit increased neurogenesis.

In another study, neural precursor cells were isolated from post-mortem Alzheimer patients and propagated in proliferative medium [71]. It was shown that isolated precursor cells could be differentiated into TuJ-1-positive neurons and GFAP-positive astrocytes. The viability of the neural precursor cells harvested from Alzheimer patients, however, was significantly lower than in an age-matched control group. Furthermore, these cells exhibited earlier senescence, characterized by discontinuation of proliferation after 2 weeks of initial plating compared to 5 weeks in the control group.

Progenitor cell activity in the SVZ of AD brains has been examined using markers such as Nestin and Musashi1 [72]. A ninefold decrease in Musashi1 immunoreactivity in the SVZ of patients with AD was seen in comparison to control brains. On the other hand, the number of GFAP- and nestin-positive cells was significantly increased in immunohistochemical studies. Additionally, a potential decrease in progenitor cell numbers was associated with cortical cholinergic loss. Thus, AD may cause depletion of subependymal progenitor cells by pushing their cell fate to immature neurons or glial cells. It is plausible that decreased activity of NSCs in the SVZ of AD brains decreases the potential for spontaneous replacement of lost neurons in the cerebral cortex of AD brain.

Neurogenesis in Down syndrome

Neurogenesis has also been investigated in Down syndrome since these patients typically present with AD by 40–50 years of age [73]. Neurospheres were isolated from post-abortion fetal tissue, grown for 10 weeks and then plated for differentiation. Considerably fewer neurons emerged after *in vitro* differentiation of neurospheres derived from the brain of Down syndrome patients compared to controls while the numbers of glial cells remained unchanged. It is unclear though whether the number of neurons generated from these neurospheres was decreased due to increased apoptosis or due to a preferential differentiation of neurosphere cells into glia.

Effect of AD on neurogenesis in cell culture models

In vitro effect of $A\beta_{42}$ on proliferation and differentiation of human NSCs

Several investigators have looked at the effects of $A\beta_{42}$ on human neural progenitor cell proliferation *in vitro*. Since Aβ induces caspase-mediated apoptosis in mature neurons [74–76], the question arises whether it has similar effects on human neural progenitor cells. Indeed, aggregated $A\beta_{42}$ was found to be toxic to human NPCs derived from cortical fetal tissue at a concentration of 1 μM *in vitro*, increasing the percentage of apoptotic cells threefold in one study [77]. The induced apoptosis could be blocked with a caspase inhibitor, showing that $A\beta_{42}$ seems to play a role in activating the caspase signaling cascade to promote apoptosis.

An impaired calcium homeostasis was thought to promote progenitor cell death by a mechanism that had been proposed earlier for the death of mature neurons [78]. Aggregated $A\beta_{42}$ was shown to increase the basal and ATP-triggered intracellular calcium concentration. Cell death could, however, be blocked by inhibiting calcium release from the IP_3- or ryanodine-responsive stores. Thus, aggregated $A\beta_{42}$ could cause progenitor cell death by promoting the accumulation of a toxic intracellular calcium concentration. A disruptive effect of aggregated $A\beta_{42}$ on neuronal differentiation of human neural progenitor cells was also seen *in vitro*. Neurospheres that had been exposed to $A\beta_{42}$ in concentrations ranging from 10 to 100 nM for 5 days showed a significantly lower number of NeuN-positive cells compared to control neurospheres.

The human data were obtained with $A\beta_{42}$ peptide that had been aggregated overnight. However, as mentioned above, different preparations of $A\beta_{42}$ have been shown to have differing effects on the viability of mature neurons [55]. Two studies therefore paid close attention to the molecular form of $A\beta_{42}$ in rodent cell culture models and, interestingly, came to a different conclusion about the effect of $A\beta_{42}$ on neural progenitor cells [79, 80]. $A\beta_{42}$ aggregated for 3 days caused a significant increase in neurogenesis in neural progenitor cells derived from E15 rat striata and P0 Bl6 mice hippocampi. This effect could not be reproduced with non-aggregated $A\beta_{42}$. Using the standardized protocol of Aβ preparation mentioned above [55], only oligomeric $A\beta_{42}$ was found to increase neurogenesis, while the fibrillary form of $A\beta_{42}$ had no significant impact on neurogenesis and inhibited proliferation of neural progenitor cells compared to the control.

Recently the effects of monomeric, oligomeric and fibrillary $A\beta_{42}$ on rodent adult NSCs were systematically studied in a rodent cell culture model [79].

Table 1. *In vitro* experiments investigating the effect of different preparations of $A\beta_{42}$ on neurogenesis.

Authors	Cell type	Aβ42 aggregation	Neurogenesis	Progenitor proliferation
Haughey et al. [77]	human NPCs	overnight	↓	↓
Lopez-Toledano and Shelanski [80]	rodent striata and hippocampi	oligomeric, aggregated	↑	↔
Lopez-Toledano and Shelanski [80]	rodent striata and hippocampi	fibrillar	↔	trend towards decrease
Heo et al. [79]	SVZ of male C57BL6 mice	monomeric	↓	↔
Heo et al. [79]	SVZ of male C57BL6 mice	oligomeric	↑	↔
Heo et al. [79]	SVZ of male C57BL6 mice	fibrillar	↔	↓
Uchida et al. [88]	cerebral cortices of E17 rats	non-aggregated	promoted apoptosis of Mash-1-positive immature neurons by down-regulation of Olig2	promoted early neuronal differentiation by up-regulation of Mash-1

Interestingly, monomeric $A\beta_{42}$ showed no significant effect on NSC proliferation and differentiation at 1 μmol/l but inhibited proliferation and neurogenesis at higher concentrations. Fibrillary $A\beta_{42}$ led to a decrease in the total number of cultured neural progenitor cells at higher concentrations. Again, oligomeric $A\beta_{42}$ was shown to enhance the proliferation and differentiation of NSCs.

What are the differences in these studies and how can the discrepant results between the human and rodent data be reconciled? One possibility is that different tissue sources might have contributed to the variant observations. On the other hand, different preparations of $A\beta_{42}$ might have played a major role in obtaining different effects on neurogenesis and progenitor cell proliferation. For example, in the human study, $A\beta_{42}$ was only aggregated overnight while it was aggregated for 3 days in one rodent study [77, 80]. Thus, the fraction of monomeric $A\beta_{42}$ might have been higher in the human cell culture experiments. This could have led to decreased neurogenesis and impaired progenitor proliferation as similarly observed for monomeric $A\beta_{42}$ at concentrations of 5 μM or higher in rodents [79].

In conclusion, only oligomeric $A\beta_{42}$ was found to be neurogenic, while monomeric and fibrillary $A\beta_{42}$ were not shown to increase neurogenesis or progenitor cell proliferation in cell culture experiments (Table 1 summarizes all results). Since fibrillary $A\beta_{42}$ is the prevalent aggregation state in the end-stage Alzheimer brain, one potential mechanism might involve decreased neurogenesis and NSC proliferation at the final stages of the disease, while the formation of $A\beta_{42}$ oligomers at earlier stages may transiently promote the initial steps of neurogenesis.

In vitro effect of APP on human NSC differentiation

Soluble APP (sAPP) has also been implicated in hampering neurogenesis in AD by shifting the cell fate of human NSCs to a glial as opposed to a neuronal lineage decision. Treatment of human NSCs with sAPP dose-dependently increased the population of GFAP-positive cells from an average of 45 % in controls (no sAPP) to an average of 83 % with the highest concentration of sAPP treatment [81]. Because apoptosis-related gene expression was not observed in human NSCs after sAPP treatment, neuronal apoptosis does not seem to be the cause of increased percentages of glia. In addition to its effect on neural lineage determination, sAPP also promoted the differentiation of human NSCs *in vitro*, an effect that was reversible by administering an antibody against the growth-factor-like domain of APP.

Role of fibroblast growth factor-2 and neurotrophic factors

Since the levels of fibroblast growth factor 2 (FGF-2) are up-regulated in the hippocampus of AD patients [82, 83] while the levels of several neurotrophic factors are down-regulated [84, 85], the hypothesis that a strong mitogen such as FGF-2 prevents neuronal differentiation and maturation in the absence of other neurotrophic factors was investigated *in vitro* [86]. Adult rat hippocampal progenitors were isolated and cultured for 7 days with different concentrations of FGF-2. The cultures treated with the highest concentration of FGF-2 showed the most prominent rise in immature and dividing cells whereas the number of mature neuronal cells strongly decreased in this setting. The FGF-2 treatment did not affect the glial lineage determination, only differentiation into neu-

Table 2. *In vitro* experimental designs of selected studies investigating neurogenesis in AD.

Authors	Cell type	Experimental setup	Neurogenesis	Progenitor proliferation
Lovell et al. [71]	post-mortem AD neurospheres	proliferation and differentiation	neurons could be generated	earlier senescence
Bahn et al. [73]	Down syndrome fetal neurospheres	proliferation and differentiation	decreased neuronal differentiation	not assessed
Kwak et al. [81]	human NSCs	treatment with sAPP	decreased by shift to glial lineage	no increased apoptosis
Chen et al. [86]	adult rat hippocampal progenitors	treatment with FGF2 and CTNF, GDNF, IGF-1	FGF-2 prevents neuronal maturation	increased

rons was found to be decreased. When treated with neurotrophic factors such as ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF) and insulin-like growth factor-1 (IGF-1), the neuronal differentiation was rescued, demonstrated by a rise in the number of TuJ-1-positive cells. The neurogenic activity of CNTF was shown to be neutralized with an antibody to CNTF. This *in vitro* model demonstrated that elevated levels of FGF-2 in AD may prevent neuronal differentiation and maturation in the absence of other neurotrophic factors. This is in contrast to the increased neurogenesis observed in the naive aging hippocampus following exogenous FGF-2 administration [87]. Thus, it appears that factors that promote neuronal differentiation are critical for improving hippocampal neurogenesis in AD. Table 2 summarizes the experimental design of the discussed cell culture experiments.

Role of basic helix-loop-helix transcription factors

To elucidate the effects of $A\beta_{42}$ on the gene expression profile of neural progenitor cells, neurospheres from E17 rat cerebral cortices were grown for 7 days, and their total RNA was hybridized to cDNA macroarrays after neurosphere dissociation and plating [88]. It was shown that $A\beta_{42}$ up-regulates mammalian achaete-schute homolog-1 (Mash-1) while Olig2 was down-regulated. The amount of increase in Mash-1 expression was found to be independent of the aggregation state of $A\beta_{42}$. Subsequently, the effects of Mash-1 over-expression and Olig2 down-regulation were assessed by using over-expression constructs and siRNA *in vitro*. Both Mash-1 over-expression and Olig2 silencing were found to promote neuronal differentiation by increasing the expression of the neuronal marker β-tubulin-III. However, in the setting of $A\beta_{42}$-induced Olig2 depletion, Mash-1 over-expression led to apoptosis. Thus, down-regulation of Olig2 and over-expression of Mash-1 in neural progenitor cells by $A\beta_{42}$ may switch the cell fate from neuronal differentiation to cell death.

Possible role for seladin-1 in neural stem cell vulnerability in AD

Seladin-1 is a gene that has been implicated in conferring resistance to AD-associated neurodegeneration and shown to be down-regulated in AD [89]. Human mesenchymal stem cells (hMSCs) derived from bone marrow biopsies were found to have high levels of seladin-1 [90]. As soon as the hMSCs were differentiated into neuronal-like phenotypes, the intracellular concentration of seladin-1 decreased significantly. High concentrations of seladin-1 were also found in the normal adult hippocampus which led the authors to conclude that this gene is primarily expressed by neural progenitors. However, no proof for their hypothesis was given. Decreased expression of seladin-1 in neural progenitors in AD might make these cells more susceptible to oxidative stress and cell death.

Effect of AD in animal models

Increased birth of immature neurons in the GCL

Another clue regarding the controversy between increased or decreased neurogenesis in AD comes from AD mouse models. Supporting the post-mortem data of increased neurogenesis, a greater density of immature neurons positive for DCX could be demonstrated in the outer granule cell layer of PDAPP mice compared to wild-type mice [91]. Interestingly, although there was an increased proliferation of progenitors, fewer of these neurons survived after 4 weeks with respect to the control. There was a trend toward increased apoptosis of cells in the outer granule cell layer, although the numbers were not significant. The percentage of progenitors that had undergone differentiation into mature neurons as shown by dual immunofluorescence for BrdU and NeuN was, however, unchanged between wild-type and the disease model, demonstrating that there was no change in the cell fate decision of neural progenitors in the setting of APP over-expression.

Looking at the SGZ, an age-dependent decrease in neurogenesis was seen which outweighed the changes in the outer granule cell layer. Therefore, impaired neurogenesis was considered the overall predominant pathology in this mouse model of AD.

The decrease in neurogenesis was, however, not associated with an age-dependent loss of dentate granule neurons. Furthermore, abnormal maturation of BrdU-positive cells into neurons was demonstrated based on their irregular shapes and perpendicular rather than parallel orientation to the GCL. Punctuate BrdU staining which is typical for cell differentiation and maturation was also observed to be decreased [92].

The investigators who had shown increased neurogenesis in AD post-mortem specimens validated their findings in mice carrying both the Indiana and Swedish double-mutations [93]. They were again able to show an increase in immature neuronal cells by staining for DCX and NeuroD. Thus, AD may promote the generation of early, immature neurons in the dentate gyrus.

Impaired neurogenesis in PS-1 and APP mutant mice

The effect of PS-1 was tested in a mouse model *in vivo* by neuron-specific over-expression of wild-type PS-1 or the P117L mutant form found in familial AD [94]. Both PS-1 constructs significantly decreased the number of BrdU-positive cells in the dentate gyrus in the transgenic mice compared to wild-type mice. Over-expression of human wild-type, but not FAD mutant PS-1, however, was shown to promote the generation of Toad64- and β-III-tubulin-labeled early post-mitotic neurons. Thus, PS-1 was demonstrated in this transgenic model to enhance the survival and differentiation of neural progenitor cells.

PS-1 FAD mutant mice were then subsequently shown to have impaired neurogenesis [95]. Fewer BrdU-labeled cells became new neurons in the hippocampus of P117L FAD mutant transgenic animals, as demonstrated by a decrease in the number of β-III-tubulin-labeled early post-mitotic neurons and calbindin-labeled mature granule cells. Mutant and wild-type mice showed, though, similar numbers of TUC-4- and PSA-NCAM-immunoreactive immature neurons. Thus, mutant PS-1 might block the maturation and survival of newly generated immature neurons in the adult rodent hippocampus.

Consistent with these data, decreased neurogenesis was also observed in another study of mutant PS-1 mice with double-staining for BrdU and a mature neuronal marker (NeuN) [96]. A study in 12 to 14-month-old mice over-expressing a mutant form of APP (the Swedish mutation APP695) demonstrated decreased neurogenesis as well [77].

Recently, there has been more evidence that AD impairs the maturation of neuronal progenitors into neurons [97]. Short-term survival of cells, assessed by BrdU labeling for 12 days, was not found to be altered in an APP/PS-1 mouse model in the dentate gyrus. However, after 30 days, a significantly lower fraction of BrdU-positive cells co-expressed the mature neuronal marker NeuN in APP single-transgenic and APP/PS1 double-transgenic mice. Thus, progenitor proliferation was not found to be impaired, but fewer progenitors managed to differentiate into mature neurons compared to control mice in this AD model.

Other experiments with APP/PS-1 mice have shown evidence for an earlier disruption of hippocampal neurogenesis [98]. Decreased neurogenesis was reported in mice carrying targeted mutations in both APP and PS-1. These 'double knock-in' mice developed aging- and region-dependent amyloid deposition starting around 6 months, and by 9 months displayed activated microglial cells coupled with amyloid. By 9 months of age, there was also a three-fold decrease in the number of putative NSCs positive for the marker mini chromosome maintenance protein 2 (MCM2) [99], and a two-fold decrease in the number of DCX-positive immature neurons. Furthermore, the decline observed in dentate neurogenesis at 9 months persisted at 18 months of age. Impaired neurogenesis was also evident through quantitative Western blot analysis of DCX content in this study. However, the decrease was restricted to the hippocampus, as the olfactory bulb neurogenic system showed no changes. A summary of the work on neurogenesis in AD animal models and post-mortem specimens is given in Table 3.

Possible model for the effect of AD on neurogenesis

Many experimental studies have been performed in recent years to elucidate the effect of AD on neurogenesis. Some of the studies at first seem to be in disagreement. However, considering that investigators studied different post-mortem patient populations, cell culture and animal models and used the term neurogenesis with respect to different stages of neuronal maturation, a common theme can be recognized in the majority of the works published. Most studies suggest that neural progenitor proliferation in the dentate gyrus is not impaired, and progenitor cells are able to differentiate into early neuronal stages. The pathology seems to come into play when these immature neurons try to accomplish the steps necessary to differentiate into mature neurons. AD might inhibit the maturation of these early non-functional neurons, leading to a decrease in the number of new

Table 3. Immunohistochemical studies investigating the effect of AD on neurogenesis in the dentate gyrus using mature and immature neuronal markers.

Authors	Study subject	Neurogenesis	Marker
Jin et al. [66]	post-mortem	↑	TUC-4, DCX
Boekhoorn et al. [70]	post-mortem	↔	Ki-67, DCX, GFAP, VWF
Jin et al. [93]	PDGF-APP$_{(Swe, Ind)}$ mice	↑	DCX, NeuroD, BrdU
Donovan et al. [91]	PDAPP mice – oGCL	↑	DCX, DCX-BrdU
Donovan et al. [91]	PDAPP mice – SGZ	↓	DCX
Wen et al. [95]	PS-1 mutant mice	↔	TUC-4, PSA-NCAM
Wen et al. [95]	PS-1 mutant mice	↓	β-III tubulin, calbindin
Wang et al. [96]	PS-1 mutant mice	↓	BrdU, NeuN
Verret et al. [97]	APP/PS-1 mutant mice	↔	BrdU
Verret et al. [97]	APP/PS-1 mutant mice	↓	NeuN
Zhang et al. [98]	APP/PS-1 mutant mice	↓	MCM2, DCX

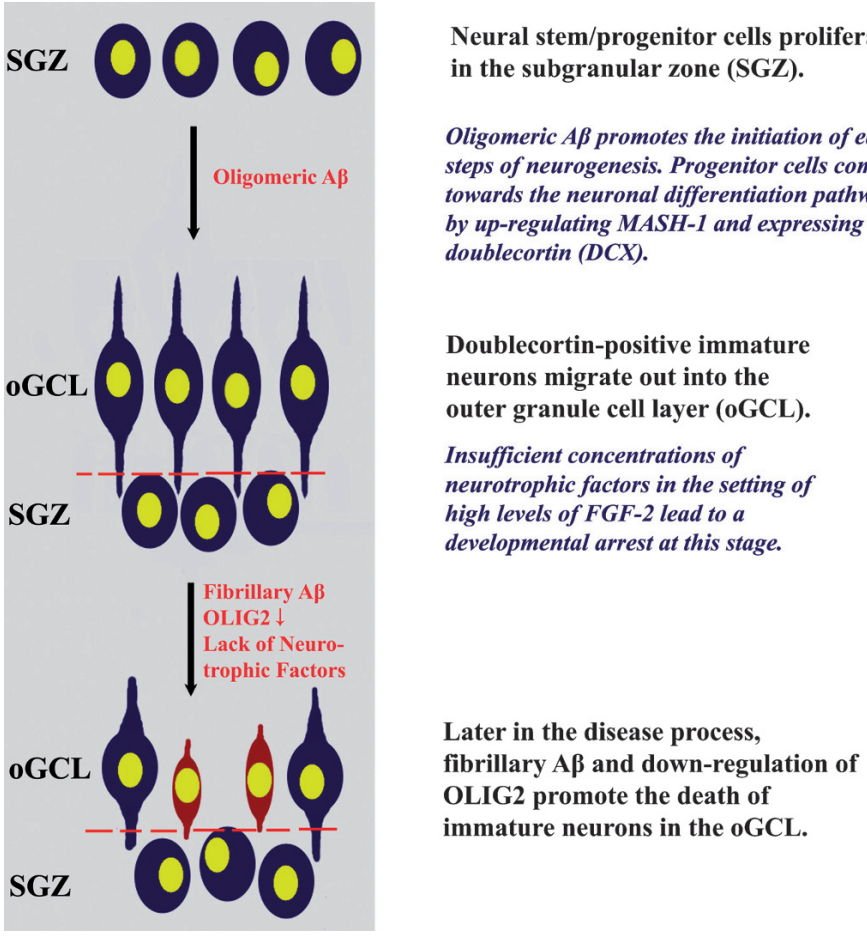

Figure 3. Possible model illustrating how neurogenesis may be affected by AD.

neurons that integrate into the functional hippocampal circuitry. Figure 3 summarizes some of the work done in the field showing possible mechanisms by which neuronal maturation is inhibited.

Considering the above, the potential NSC-based therapies for AD need to focus on providing a suitable microenvironment for differentiation of the progeny of NSCs into functional neurons that incorporate appropriately into the brain circuitry. Stimulating the proliferation of endogenous NSCs without additional treatment for neuronal differentiation of newly born cells might not improve the patient's condition. Additionally, as neurogenesis is limited to certain regions such as the dentate gyrus of the hippocampus and SVZ

lining the ventricles, significant replacement of lost neurons in AD through activation of endogenous NSCs may not be feasible. For example, though some neurogenesis by SVZ NSCs lining the hippocampus has been reported for the CA1 and CA3 regions of neonatal and young animals following certain types of injury or neurodegeneration [100, 101], there is no clear evidence so far for such neurogenesis in the adult hippocampus following similar neurodegeneration. Additionally, new neurons generated from NSCs in the adult dentate gyrus following various types of hippocampal injury have not shown a capability for either migrating into the CA1 or CA3 regions or differentiating into hippocampal CA1 or CA3 pyramidal neurons. Rather, the phenotypic differentiation of new neurons generated from NSCs of the dentate gyrus is restricted to the formation of mostly dentate granule cells and some interneurons [102, 103]. Thus, the extent of neurogenesis in the dentate gyrus is unlikely to have any influence on CA1 neuronal survival in AD, even though the CA1 subfield is the hot spot of neurodegeneration in AD. From this perspective, grafting of NSCs into specific regions of the brain appears critical for replenishing the lost neurons in AD.

Nevertheless, transplantation of NSCs without additional treatment for priming the microenvironment may not improve the patient's condition because grafted NSCs are unable to differentiate fully into mature neurons in the hostile microenvironment prevailing in the AD brain. One of the keys to AD pathology may lie in the impaired differentiation of neuronal progenitors which cannot be addressed by cell transplantation alone. Infusion of neurotrophic factors or drugs capable of promoting the final steps of neuronal differentiation seems to be a valid approach for increasing neurogenesis in the AD brain based on the data summarized in this review. Indeed, infusions of nerve growth factor in aged animal models have been shown to increase cognitive function [104, 105]. A phase 1 clinical trial of nerve growth factor gene therapy for AD has been performed which showed that it is safe to transplant fibroblasts genetically engineered to express NGF into the forebrain [106]. Moreover, mini-mental status examination of the transplanted patients suggested a slowing in the rate of cognitive decline. Other strategies that may be beneficial for enhancing neuronal differentiation and survival in AD include exposure to an enriched environment in the form of new learning and cognitive exercise, simple physical exercise such as running and administration of neuronal differentiation factors such as BDNF. In fact, studies in animal models have shown increased neurogenesis in the naive aged hippocampus and/or the hippocampus with AD pathology following exposure to an enriched environment [107–110] and physical exercise [9]. Similarly, peripheral administration of BDNF has been found to increase neurogenesis in the injured aged hippocampus [111]. Once a suitable microenvironment permissive for neuronal differentiation has been created, cell replacement therapies may play a role in replenishing lost neurons in a second treatment step. Grafting of NSCs or neurons derived from NSCs *in vitro* appears imminent if the hypothesis turns out be correct that the pools of endogenous NSCs in the AD brain exhibit depletion in number through premature early differentiation. Thus, NSC grafting may be important not only for replacing the degenerated neurons but also for restoring the pool of cycling progenitors so that significant neurogenesis continues even at later stages of the disease.

Furthermore, additional studies are required to address fully the complexity of AD with respect to neurogenesis. Current research has mostly focused on the response of neurogenesis to Aβ. Other disease models need to be investigated that integrate the complexity of the disease, including neurofibrillary tangles, dystrophic neuritis, increased cytokine activation and impairments in mitochondrial function. Since new animal models have been developed that more closely mimic AD pathology [54], the future holds promise that we will gain further insight into the impact of AD on neurogenesis.

Acknowledgments. B.W. was supported by the Congress of Neurological Surgeons (CNS) Basic/Translational Resident Research Fellowship Award. This work was also supported by grants from the Department of Veterans Affairs (VA Merit Review Award to A.K.S.), National Institute for Aging (AG20924 to A.K.S.), and National Institute of Neurological Disorders and Stroke (NS054780 & NS 043507 to A.K.S.).

1 Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y., Jorm, A., Mathers, C., Menezes, P. R., Rimmer, E. and Scazufca, M. (2005) Global prevalence of dementia: a Delphi consensus study. Lancet 366, 2112–2117.
2 Hebert, L. E., Scherr, P. A., Bienias, J. L., Bennett, D. A. and Evans, D. A. (2003) Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch. Neurol. 60, 1119–1122.
3 Mohs, R. C., Schmeidler, J. and Aryan, M. (2000) Longitudinal studies of cognitive, functional and behavioural change in patients with Alzheimer's disease. Stat. Med. 19, 1401–1409.
4 Gage, F. H. (2000) Mammalian neural stem cells. Science 287, 1433–1438.
5 Reynolds, B. A. and Weiss, S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710.
6 Abrous, D. N., Koehl, M. and Le Moal, M. (2005) Adult neurogenesis: from precursors to network and physiology. Physiol. Rev. 85, 523–569.
7 Gould, E. (2007) How widespread is adult neurogenesis in mammals? Nat Rev Neurosci. 8, 481–488.
8 van Praag, H., Christie, B. R., Sejnowski, T. J. and Gage, F. H. (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc. Natl. Acad. Sci. USA 96, 13427–13431.

9 van Praag, H., Shubert, T., Zhao, C. and Gage, F. H. (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. J. Neurosci. 25, 8680–8685.
10 Kempermann, G., Kuhn, H. G. and Gage, F. H. (1998) Experience-induced neurogenesis in the senescent dentate gyrus. J. Neurosci. 18, 3206–3212.
11 Parent, J. M., Yu, T. W., Leibowitz, R. T., Geschwind, D. H., Sloviter, R. S. and Lowenstein, D. H. (1997) Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J. Neurosci. 17, 3727–3738.
12 Jin, K., Minami, M., Lan, J. Q., Mao, X. O., Batteur, S., Simon, R. P. and Greenberg, D. A. (2001) Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc. Natl. Acad. Sci. USA 98, 4710–4715.
13 Hattiangady, B. and Shetty, A. K. (2008) Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus. Neurobiol. Aging 29, 129–147.
14 Hagg, T. (2007) Endogenous regulators of adult CNS neurogenesis. Curr. Pharm. Des. 13, 1829–1840.
15 Gould, E. and Tanapat, P. (1999) Stress and hippocampal neurogenesis. Biol. Psychiatry 46, 1472–1479.
16 Kuhn, H. G., Dickinson-Anson, H. and Gage, F. H. (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. 16, 2027–2033.
17 Nacher, J., Alonso-Llosa, G., Rosell, D. R. and McEwen, B. S. (2003) NMDA receptor antagonist treatment increases the production of new neurons in the aged rat hippocampus. Neurobiol. Aging 24, 273–284.
18 Rao, M. S., Hattiangady, B., Abdel-Rahman, A., Stanley, D. P. and Shetty, A. K. (2005) Newly born cells in the ageing dentate gyrus display normal migration, survival and neuronal fate choice but endure retarded early maturation. Eur. J. Neurosci. 21, 464–476.
19 Rao, M. S., Hattiangady, B. and Shetty, A. K. (2006) The window and mechanisms of major age-related decline in the production of new neurons within the dentate gyrus of the hippocampus. Aging Cell 5, 545–558.
20 Hattiangady, B., Rao, M. S. and Shetty, A. K. (2004) Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. Neurobiol. Dis. 17, 473–490.
21 Gould, E., Beylin, A., Tanapat, P., Reeves, A. and Shors, T. J. (1999) Learning enhances adult neurogenesis in the hippocampal formation. Nat. Neurosci. 2, 260–265.
22 Aimone, J. B., Wiles, J. and Gage, F. H. (2006) Potential role for adult neurogenesis in the encoding of time in new memories. Nat. Neurosci. 9, 723–727.
23 Leuner, B., Gould, E. and Shors, T. J. (2006) Is there a link between adult neurogenesis and learning? Hippocampus 16, 216–224.
24 Sahay, A. and Hen, R. (2007) Adult hippocampal neurogenesis in depression. Nat Neurosci. 10, 1110–1115.
25 Alzheimer, A. (1907) Ueber eine eigenartige Erkrankung der Hirnrinde. Allg. Z. Psychiatrie Psychisch-Gerichtl. Med. 64, 146–148.
26 Alzheimer, A. (1911) Ueber eigenartige Krankheitsfaelle des spaeteren Alters. Zbl. Ges. Neurol. Psych. 4, 356–385.
27 Glenner, G. G. and Wong, C. W. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. 120, 885–890.
28 Eckman, C. B. and Eckman, E. A. (2007) An update on the amyloid hypothesis. Neurol. Clin. 25, 669–682, vi.
29 Jarrett, J. T., Berger, E. P. and Lansbury, P. T., Jr. (1993) The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Biochemistry 32, 4693–4697.
30 Vassar, R. and Citron, M. (2000) Abeta-generating enzymes: recent advances in beta- and gamma-secretase research. Neuron 27, 419–422.
31 Cai, X. D., Golde, T. E. and Younkin, S. G. (1993) Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science 259, 514–516.
32 Kim, J., Onstead, L., Randle, S., Price, R., Smithson, L., Zwizinski, C., Dickson, D. W., Golde, T. and McGowan, E. (2007) Abeta40 inhibits amyloid deposition in vivo. J. Neurosci. 27, 627–633.
33 Murrell, J., Farlow, M., Ghetti, B. and Benson, M. D. (1991) A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science 254, 97–99.
34 Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L. and et al. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349, 704–706.
35 Chartier-Harlin, M. C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J. and et al. (1991) Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. Nature 353, 844–846.
36 Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B. and Lannfelt, L. (1992) A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. Nat. Genet. 1, 345–347.
37 Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I. and Selkoe, D. J. (1992) Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. Nature 360, 672–674.
38 Felsenstein, K. M., Hunihan, L. W. and Roberts, S. B. (1994) Altered cleavage and secretion of a recombinant beta-APP bearing the Swedish familial Alzheimer's disease mutation. Nat. Genet. 6, 251–255.
39 Suzuki, N., Cheung, T. T., Cai, X. D., Odaka, A., Otvos, L., Jr., Eckman, C., Golde, T. E. and Younkin, S. G. (1994) An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. Science 264, 1336–1340.
40 De Strooper, B. (2003) Aph-1, Pen-2, and nicastrin with presenilin generate an active gamma-secretase complex. Neuron 38, 9–12.
41 Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T. and Selkoe, D. J. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 398, 513–517.
42 Borchelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitsky, T., Prada, C. M., Kim, G., Seekins, S., Yager, D., Slunt, H. H., Wang, R., Seeger, M., Levey, A. I., Gandy, S. E., Copeland, N. G., Jenkins, N. A., Price, D. L., Younkin, S. G. and Sisodia, S. S. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1–42/1–40 ratio *in vitro* and in vivo. Neuron 17, 1005–1013.
43 Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., Johnson-Wood, K., Lee, M., Seubert, P., Davis, A., Kholodenko, D., Motter, R., Sherrington, R., Perry, B., Yao, H., Strome, R., Lieberburg, I., Rommens, J., Kim, S., Schenk, D., Fraser, P., St George Hyslop, P. and Selkoe, D. J. (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. Nat. Med. 3, 67–72.
44 Jankowsky, J. L., Fadale, D. J., Anderson, J., Xu, G. M., Gonzales, V., Jenkins, N. A., Copeland, N. G., Lee, M. K., Younkin, L. H., Wagner, S. L., Younkin, S. G. and Borchelt, D. R. (2004) Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. Hum. Mol. Genet. 13, 159–170.
45 Jankowsky, J. L., Younkin, L. H., Gonzales, V., Fadale, D. J., Slunt, H. H., Lester, H. A., Younkin, S. G. and Borchelt,

D. R. (2007) Rodent A beta modulates the solubility and distribution of amyloid deposits in transgenic mice. J. Biol. Chem. 282, 22707–22720.
46 Duyckaerts, C., Potier, M. C. and Delatour, B. (2008) Alzheimer disease models and human neuropathology: similarities and differences. Acta Neuropathol. 115, 5–38.
47 Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F. and et al. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. Nature 373, 523–527.
48 Irizarry, M. C., Soriano, F., McNamara, M., Page, K. J., Schenk, D., Games, D. and Hyman, B. T. (1997) Abeta deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. J. Neurosci. 17, 7053–7059.
49 Borchelt, D. R., Ratovitski, T., van Lare, J., Lee, M. K., Gonzales, V., Jenkins, N. A., Copeland, N. G., Price, D. L. and Sisodia, S. S. (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron 19, 939–945.
50 Janus, C., D'Amelio, S., Amitay, O., Chishti, M. A., Strome, R., Fraser, P., Carlson, G. A., Roder, J. C., St George-Hyslop, P. and Westaway, D. (2000) Spatial learning in transgenic mice expressing human presenilin 1 (PS1) transgenes. Neurobiol Aging 21, 541–549.
51 Radde, R., Bolmont, T., Kaeser, S. A., Coomaraswamy, J., Lindau, D., Stoltze, L., Calhoun, M. E., Jaggi, F., Wolburg, H., Gengler, S., Haass, C., Ghetti, B., Czech, C., Holscher, C., Mathews, P. M. and Jucker, M. (2006) Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. EMBO Rep. 7, 940–946.
52 Garcia-Alloza, M., Robbins, E. M., Zhang-Nunes, S. X., Purcell, S. M., Betensky, R. A., Raju, S., Prada, C., Greenberg, S. M., Bacskai, B. J. and Frosch, M. P. (2006) Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. Neurobiol. Dis. 24, 516–524.
53 Lee, M. K., Borchelt, D. R., Kim, G., Thinakaran, G., Slunt, H. H., Ratovitski, T., Martin, L. J., Kittur, A., Gandy, S., Levey, A. I., Jenkins, N., Copeland, N., Price, D. L. and Sisodia, S. S. (1997) Hyperaccumulation of FAD-linked presenilin 1 variants in vivo. Nat. Med. 3, 756–760.
54 Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., Metherate, R., Mattson, M. P., Akbari, Y. and LaFerla, F. M. (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39, 409–421.
55 Dahlgren, K. N., Manelli, A. M., Stine, W. B., Jr., Baker, L. K., Krafft, G. A. and LaDu, M. J. (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. J. Biol. Chem. 277, 32046–32053.
56 Kuo, Y. M., Emmerling, M. R., Vigo-Pelfrey, C., Kasunic, T. C., Kirkpatrick, J. B., Murdoch, G. H., Ball, M. J. and Roher, A. E. (1996) Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. J. Biol. Chem. 271, 4077–4081.
57 Naslund, J., Haroutunian, V., Mohs, R., Davis, K. L., Davies, P., Greengard, P. and Buxbaum, J. D. (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. JAMA 283, 1571–1577.
58 Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T. and Hyman, B. T. (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 42, 631–639.
59 Bussiere, T., Friend, P. D., Sadeghi, N., Wicinski, B., Lin, G. I., Bouras, C., Giannakopoulos, P., Robakis, N. K., Morrison, J. H., Perl, D. P. and Hof, P. R. (2002) Stereologic assessment of the total cortical volume occupied by amyloid deposits and its relationship with cognitive status in aging and Alzheimer's disease. Neuroscience 112, 75–91.
60 Klein, W. L., Krafft, G. A. and Finch, C. E. (2001) Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci. 24, 219–224.
61 Klein, W. L., Stine, W. B., Jr. and Teplow, D. B. (2004) Small assemblies of unmodified amyloid beta-protein are the proximate neurotoxin in Alzheimer's disease. Neurobiol. Aging 25, 569–580.
62 Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J. and Selkoe, D. J. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.
63 Lesne, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., Gallagher, M. and Ashe, K. H. (2006) A specific amyloid-beta protein assembly in the brain impairs memory. Nature 440, 352–357.
64 Kim, S. H., Tang, Y. P. and Sisodia, S. S. (2006) Abeta star: a light onto synaptic dysfunction? Nat Med 12, 760–1; discussion 761.
65 Lee, E. B., Leng, L. Z., Zhang, B., Kwong, L., Trojanowski, J. Q., Abel, T. and Lee, V. M. (2006) Targeting amyloid-beta peptide (Abeta) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Abeta precursor protein (APP) transgenic mice. J. Biol. Chem. 281, 4292–4299.
66 Jin, K., Peel, A. L., Mao, X. O., Xie, L., Cottrell, B. A., Henshall, D. C. and Greenberg, D. A. (2004) Increased hippocampal neurogenesis in Alzheimer's disease. Proc. Natl. Acad. Sci. USA 101, 343–347.
67 Yang, Y., Mufson, E. J. and Herrup, K. (2003) Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease. J. Neurosci. 23, 2557–2563.
68 Phillips, W., Michell, A. W. and Barker, R. A. (2006) Neurogenesis in diseases of the central nervous system. Stem Cells Dev. 15, 359–379.
69 Jin, K., Xie, L., Mao, X. O. and Greenberg, D. A. (2006) Alzheimer's disease drugs promote neurogenesis. Brain Res. 1085, 183–188.
70 Boekhoorn, K., Joels, M. and Lucassen, P. J. (2006) Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. Neurobiol. Dis. 24, 1–14.
71 Lovell, M. A., Geiger, H., Van Zant, G. E., Lynn, B. C. and Markesbery, W. R. (2006) Isolation of neural precursor cells from Alzheimer's disease and aged control post-mortem brain. Neurobiol. Aging 27, 909–917.
72 Ziabreva, I., Perry, E., Perry, R., Minger, S. L., Ekonomou, A., Przyborski, S. and Ballard, C. (2006) Altered neurogenesis in Alzheimer's disease. J. Psychosom. Res. 61, 311–316.
73 Bahn, S., Mimmack, M., Ryan, M., Caldwell, M. A., Jauniaux, E., Starkey, M., Svendsen, C. N. and Emson, P. (2002) Neuronal target genes of the neuron-restrictive silencer factor in neurospheres derived from fetuses with Down's syndrome: a gene expression study. Lancet 359, 310–315.
74 Jordan, J., Galindo, M. F. and Miller, R. J. (1997) Role of calpain- and interleukin-1 beta converting enzyme-like proteases in the beta-amyloid-induced death of rat hippocampal neurons in culture. J. Neurochem. 68, 1612–1621.
75 Guo, Q., Sebastian, L., Sopher, B. L., Miller, M. W., Ware, C. B., Martin, G. M. and Mattson, M. P. (1999) Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid beta-peptide toxicity: central roles of superoxide production and caspase activation. J. Neurochem. 72, 1019–1029.
76 Mattson, M. P., Partin, J. and Begley, J. G. (1998) Amyloid beta-peptide induces apoptosis-related events in synapses and dendrites. Brain Res. 807, 167–176.
77 Haughey, N. J., Nath, A., Chan, S. L., Borchard, A. C., Rao, M. S. and Mattson, M. P. (2002) Disruption of neurogenesis by amyloid beta-peptide, and perturbed neural progenitor cell homeostasis, in models of Alzheimer's disease. J. Neurochem. 83, 1509–1524.

78 Mattson, M. P., Cheng, B., Davis, D., Bryant, K., Lieberburg, I. and Rydel, R. E. (1992) Beta-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J. Neurosci. 12, 376–389.
79 Heo, C., Chang, K. A., Choi, H. S., Kim, H. S., Kim, S., Liew, H., Kim, J. A., Yu, E., Ma, J. and Suh, Y. H. (2007) Effects of the monomeric, oligomeric, and fibrillar Abeta42 peptides on the proliferation and differentiation of adult neural stem cells from subventricular zone. J. Neurochem. 102, 493–500.
80 Lopez-Toledano, M. A. and Shelanski, M. L. (2004) Neurogenic effect of beta-amyloid peptide in the development of neural stem cells. J. Neurosci. 24, 5439–5444.
81 Kwak, Y. D., Brannen, C. L., Qu, T., Kim, H. M., Dong, X., Soba, P., Majumdar, A., Kaplan, A., Beyreuther, K. and Sugaya, K. (2006) Amyloid precursor protein regulates differentiation of human neural stem cells. Stem Cells Dev. 15, 381–389.
82 Cummings, B. J., Su, J. H. and Cotman, C. W. (1993) Neuritic involvement within bFGF immunopositive plaques of Alzheimer's disease. Exp. Neurol. 124, 315–325.
83 Stopa, E. G., Gonzalez, A. M., Chorsky, R., Corona, R. J., Alvarez, J., Bird, E. D. and Baird, A. (1990) Basic fibroblast growth factor in Alzheimer's disease. Biochem. Biophys. Res. Commun. 171, 690–696.
84 Durany, N., Michel, T., Kurt, J., Cruz-Sanchez, F. F., Cervas-Navarro, J. and Riederer, P. (2000) Brain-derived neurotrophic factor and neurotrophin-3 levels in Alzheimer's disease brains. Int. J. Dev. Neurosci. 18, 807–813.
85 Hock, C., Heese, K., Hulette, C., Rosenberg, C. and Otten, U. (2000) Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. Arch. Neurol. 57, 846–851.
86 Chen, H., Tung, Y. C., Li, B., Iqbal, K. and Grundke-Iqbal, I. (2007) Trophic factors counteract elevated FGF-2-induced inhibition of adult neurogenesis. Neurobiol. Aging 28, 1148–1162.
87 Rai, K. S., Hattiangady, B. and Shetty, A. K. (2007) Enhanced production and dendritic growth of new dentate granule cells in the middle-aged hippocampus following intracerebroventricular FGF-2 infusions. Eur. J. Neurosci. 26, 1765–1779.
88 Uchida, Y., Nakano, S., Gomi, F. and Takahashi, H. (2007) Differential regulation of basic helix-loop-helix factors Mash1 and Olig2 by beta-amyloid accelerates both differentiation and death of cultured neural stem/progenitor cells. J. Biol. Chem. 282, 19700–19709.
89 Greeve, I., Hermans-Borgmeyer, I., Brellinger, C., Kasper, D., Gomez-Isla, T., Behl, C., Levkau, B. and Nitsch, R. M. (2000) The human DIMINUTO/DWARF1 homolog seladin-1 confers resistance to Alzheimer's disease-associated neurodegeneration and oxidative stress. J. Neurosci. 20, 7345–7352.
90 Benvenuti, S., Saccardi, R., Luciani, P., Urbani, S., Deledda, C., Cellai, I., Francini, F., Squecco, R., Rosati, F., Danza, G., Gelmini, S., Greeve, I., Rossi, M., Maggi, R., Serio, M. and Peri, A. (2006) Neuronal differentiation of human mesenchymal stem cells: changes in the expression of the Alzheimer's disease-related gene seladin-1. Exp. Cell Res. 312, 2592–2604.
91 Donovan, M. H., Yazdani, U., Norris, R. D., Games, D., German, D. C. and Eisch, A. J. (2006) Decreased adult hippocampal neurogenesis in the PDAPP mouse model of Alzheimer's disease. J. Comp. Neurol. 495, 70–83.
92 Cameron, H. A. and McKay, R. D. (2001) Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J. Comp. Neurol. 435, 406–417.
93 Jin, K., Galvan, V., Xie, L., Mao, X. O., Gorostiza, O. F., Bredesen, D. E. and Greenberg, D. A. (2004) Enhanced neurogenesis in Alzheimer's disease transgenic (PDGF-APPSw,Ind) mice. Proc Natl Acad Sci USA 101, 13363–13367.
94 Wen, P. H., Shao, X., Shao, Z., Hof, P. R., Wisniewski, T., Kelley, K., Friedrich, V. L., Jr., Ho, L., Pasinetti, G. M., Shioi, J., Robakis, N. K. and Elder, G. A. (2002) Overexpression of wild type but not an FAD mutant presenilin-1 promotes neurogenesis in the hippocampus of adult mice. Neurobiol. Dis. 10, 8–19.
95 Wen, P. H., Hof, P. R., Chen, X., Gluck, K., Austin, G., Younkin, S. G., Younkin, L. H., DeGasperi, R., Gama Sosa, M. A., Robakis, N. K., Haroutunian, V. and Elder, G. A. (2004) The presenilin-1 familial Alzheimer disease mutant P117L impairs neurogenesis in the hippocampus of adult mice. Exp. Neurol. 188, 224–237.
96 Wang, R., Dineley, K. T., Sweatt, J. D. and Zheng, H. (2004) Presenilin 1 familial Alzheimer's disease mutation leads to defective associative learning and impaired adult neurogenesis. Neuroscience 126, 305–312.
97 Verret, L., Jankowsky, J. L., Xu, G. M., Borchelt, D. R. and Rampon, C. (2007) Alzheimer's-type amyloidosis in transgenic mice impairs survival of newborn neurons derived from adult hippocampal neurogenesis. J. Neurosci. 27, 6771–6780.
98 Zhang, C., McNeil, E., Dressler, L. and Siman, R. (2007) Long-lasting impairment in hippocampal neurogenesis associated with amyloid deposition in a knock-in mouse model of familial Alzheimer's disease. Exp. Neurol. 204, 77–87.
99 Maiorano, D., Lutzmann, M. and Mechali, M. (2006) MCM proteins and DNA replication. Curr. Opin. Cell. Biol. 18, 130–136.
100 Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T. and Nakafuku, M. (2002) Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 110, 429–441.
101 Dong, H., Csernansky, C. A., Goico, B. and Csernansky, J. G. (2003) Hippocampal neurogenesis follows kainic acid-induced apoptosis in neonatal rats. J. Neurosci. 23, 1742–1749.
102 Liu, S., Wang, J., Zhu, D., Fu, Y., Lukowiak, K. and Lu, Y. M. (2003) Generation of functional inhibitory neurons in the adult rat hippocampus. J. Neurosci. 23, 732–736.
103 Zhao, C., Deng, W. and Gage, F. H. (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132, 645–660.
104 Tuszynski, M. H., U, H. S., Amaral, D. G. and Gage, F. H. (1990) Nerve growth factor infusion in the primate brain reduces lesion-induced cholinergic neuronal degeneration. J. Neurosci. 10, 3604–3614.
105 Fischer, W., Wictorin, K., Bjorklund, A., Williams, L. R., Varon, S. and Gage, F. H. (1987) Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. Nature 329, 65–68.
106 Tuszynski, M. H., Thal, L., Pay, M., Salmon, D. P., U, H. S., Bakay, R., Patel, P., Blesch, A., Vahlsing, H. L., Ho, G., Tong, G., Potkin, S. G., Fallon, J., Hansen, L., Mufson, E. J., Kordower, J. H., Gall, C. and Conner, J. (2005) A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. Nat. Med. 11, 551–555.
107 Kempermann, G., Gast, D. and Gage, F. H. (2002) Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann. Neurol. 52, 135–143.
108 Nithianantharajah, J. and Hannan, A. J. (2006) Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat. Rev. Neurosci. 7, 697–709.
109 Levi, O. and Michaelson, D. M. (2007) Environmental enrichment stimulates neurogenesis in apolipoprotein E3 and neuronal apoptosis in apolipoprotein E4 transgenic mice. J. Neurochem. 100, 202–210.
110 Dhanushkodi, A. and Shetty, A. K. (2008) Is exposure to enriched environment beneficial for functional post-lesional recovery in temporal lobe epilepsy? Neurosci. Biobehav. Rev. 32, 657–674.
111 Hattiangady, B. and Shetty, A. K. (2007) Subcutaneous administration of BDNF dramatically enhances dentate neurogenesis in the injured aged hippocampus. SFN abstract 562.12