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Changes in the Neurogenesis and Axonal Sprouting in the Organotypic Hippocampal Slice Culture by $A\beta_{25-35}$ Treatment

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Induction of neurogenesis can occur in the hippocampus in response to various pathological conditions, such as Alzheimer's disease. The aim of this study was to investigate the changes that occur in endogenous neural stem cells in response to amyloid beta $(A\beta)_{25-35}$-induced neuronal cell damage in organotypic hippocampal slice cultures. Cresyl violet staining and Fluoro-Jade B staining were used to detect neuronal cell damage and changes of mossy fiber terminals were observed by Timm's staining. The immunofluorescence staining was used to detect the newly generated cells in the subgranular zone (SGZ) of the dentate gyrus with specific marker, 5-bromo-2′-deoxyuridine (BrdU), Ki-67, Nestin, and doublecortin (DCX). In compared to control slices, neuronal cell damage was observed and the mossy fibers were expanded to CA3 area by treatment with $A\beta_{25-35}$. Ki-67/Nestin- and BrdU/DCX-positive cells were detected in the SGZ. In conclusion, these results demonstrate that Aβ-induced neuronal damage results in an increase in endogenous neural stem cells in rat hippocampal slice cultures not only for gliosis but also for neurogenesis.

Key Words: Neurogenesis, $A\beta_{25-35}$-induced neuronal cell damage, Subgranular zone, Dentate gyrus, Mossy fiber terminals

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INTRODUCTION

Neural stem cells have the capacity to self-renew, proliferate, and give rise to lineage-restricted neuronal and/or glial progenitor cells and postmitotic specialized daughter cells. Neural progenitor cells located in the subgranular zone (SGZ) of the dentate gyrus (DG) give rise to immature neurons. These then migrate to the granule cell layer, where they differentiate into granule neurons that project to the CA3 region. Here they become electrically active, and eventually become functionally integrated into preexisting hippocampal circuits (Lie et al., 2004; Picard-Riera et al., 2004; Raineteau et al., 2004).

Adult hippocampal neurogenesis has been implicated in the regulation of cognition (Rola et al., 2004; Schaffer & Gage, 2004) and the cellular repair of neurons in the central nervous system (Lie et al., 2004; Picard-Riera et al., 2004; Ziabreva et al., 2006). Recent studies have suggested that endogenous neurodegenerative mechanisms are activated in the adult human brain in response to various neuronal injuries (Ziabreva et al., 2006). The stimulation of both the proliferation and differentiation of endogenous neural progenitor cells and the transplantation of exogenous neural progenitor cells into the brain are potential therapeutic strategies in neurodegenerative disorders, such as Alzheimer's disease (AD) (Mazur-Kolecka et al., 2006).

Amyloid beta (Aβ) peptide promotes synaptic dysfunction and neuronal cell death, leading to memory impairment (Mattson, 2000; Haughey et al., 2002). For this reason it is widely used for investigating the biochemical and molecular

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mechanisms underlying AD. Studies using cellular and mice models of AD have suggested that Aβ may play a role in the regulation of adult neurogenesis (Haydar et al., 1999; Haughey et al., 2002; Lie et al., 2004; Raineteau et al., 2004; Namba et al., 2007; Zhang et al., 2007; Wang et al., 2008). Furthermore, it has been reported that the oligomeric peptide, $A\beta_{42}$, increases the proliferation and neuronal differentiation of neural stem cells, leading to neurogenesis (Heo et al., 2007). Although it is still controversial that hippocampal neurogenesis is induced in mouse models of AD (Wang et al., 2007; Zhang et al., 2007; Rodriguez et al., 2009), several studies have reported that it is critically increased in AD patients (Ziabreva et al., 2006; Heo et al., 2007) and in AD models employing young animals (Lopez-Toledano & Shelanski, 2007; Chuang, 2010).

Organotypic hippocampal slice cultures are widely used to study cellular and molecular mechanisms underlying neurogenesis and neuronal cell death in a variety of neurophysiological states (Haydar et al., 1999; Dehghani et al., 2003; Scheffler et al., 2003; Raineteau et al., 2004; Chechneva et al., 2005; Sadgrove et al., 2006; Namba et al., 2007; Suh et al., 2008). Their use is advantageous for studying the detection of region-specific changes, induction of stimulation, and the treatment effect of drugs. $A\beta_{25-35}$ is a shorter peptide than $A\beta_{1-42}$, but it has similar endogenous effects and can induce neuronal cell death in a dose-dependent manner (Lopez-Toledano & Shelanski, 2004; Suh et al., 2008).

Thus, the aim of this study was to investigate the changes that occur in endogenous neural stem cells in response to $A\beta_{25-35}$-induced neuronal cell damage in rat organotypic hippocampal slice cultures.

MATERIALS AND METHODS

Preparation of Organotypic Hippocampal Slice Cultures and Treatment with $A\beta_{25-35}$

The organotypic hippocampal slice cultures were prepared from the hippocampus of 8-day-old Sprague Dawley rats, as described previously (Suh et al., 2008). Briefly, they were dissected and cut into slices, 400 μm in thickness, using a McIlwain tissue chopper (The Mickle Laboratory Engineering Co., Ltd, Surrey, UK). The slices were carefully placed on porous membrane filters (0.4 μm, Millicell-CM; Millipore, Bedford, MA, USA), which were then inserted into 6-well tissue culture plates. Each well was incubated in 1.2 mL of culture medium for 3 weeks at 37°C, with 95% O_2 and 5% CO_2.

$A\beta_{25-35}$ (Sigma, St. Louis, MO, USA) was applied to the culture medium of each well for 3 days, at a final concentration of 1, 2.5, and 5 μM.

Cresyl Violet Staining

The cultured slices were immersed in 0.5% cresyl violet solution for 10 min, after which the slices were sequentially washed with 50% ethanol for 1 min, 70% ethanol for 5 min, 80% ethanol for 1 min, 90% ethanol for 1 min, and 100% ethanol for 1 min. The slices were then mounted onto slides, and the images were obtained using light microscopy (Zeiss Axiovert 200; Carl Zeiss Inc., Göttingen, Germany).

Fluoro-Jade B Staining

The cultured slices were mounted onto 1% gelatin-coated slides. The slides were sequentially incubated in 100% ethanol for 3 min; 70% ethanol for 1 min; and distilled water for 1 min. The slides were then oxidized in 0.06% $KMnO_4$ solution for 15 min, followed by 3 washes with double distilled water (ddH_2O). The cultured slices were stained with a 0.004% solution of Fluoro-Jade B (Histo-Chem Inc., Jefferson, AR, USA) in 0.1% acetic acid for 30 min, after which they were washed with distilled water. The slides were mounted using DPX medium (Electron Microscopy Sciences, Ft. Washington, PA, USA) and detected at 534 nm, using fluorescence microscopy (Zeiss Axiovert 200; Carl Zeiss Inc.).

Timm's Staining

Prior to staining, the slices were immersed in a 1% sodium sulfide solution, fixed with 0.3% glutaraldehyde, and dehydrated with ethanol. For Timm's staining, silver sulfide was developed in a citrate-buffered solution in a dark room. The images were obtained using light microscopy (Zeiss Axiovert 200; Carl Zeiss Inc.).

5-Bromo-2′-deoxyuridine (BrdU) Labeling

Twenty-four hours prior to fixation, BrdU (20 μM) was added for 24 h to both control and $A\beta_{25-35}$-treated groups. The cultured slices were washed in phosphate-buffered saline (PBS) in order to remove excess BrdU and fixed in 4% paraformaldehyde (PFA). For BrdU-positive cell counting, images of the SGZ were obtained at 40× magnification. BrdU-positive cells were counted as the number of images per 200×200 μm. The NIH software program Image J, was used for morphometric measurements.

Immunofluorescence Staining

The cultured slices were fixed for 24 h with 4% PFA (Merck, Darmstadt, Germany), dissolved in 0.1 M phosphate buffer. The slices were then incubated for 1 h at room temperature in a blocking solution containing 0.2% Triton X-100 and 10% bovine serum albumin (BSA), dissolved in PBS. Slices were incubated for 24 h at 4°C in the following primary antibodies: mouse anti-BrdU (1:500, monoclonal IgG_1; Chemicon International, Temecula, CA, USA); mouse anti-Nestin (1:1000, monoclonal IgG_1; Chemicon International); and mouse anti-DCX (1:500, monoclonal IgG_1; BD Bioscience, NJ, USA). After 3 washes with 3% BSA dissolved in PBS, anti-

Table 1. Stem cell specific markers for immunofluorescence staining

Antibody	Vendor	Cell type	References
BrdU (S-phase bromo-deoxyuridine)	Chemicon International, Temecula, CA, USA	Proliferating cells	Tanvig M et al. (2009) *Brain Res.* **1295**, 1-12.
Ki-67	Novus Biologicals Inc., Littleton, CO, USA	Cell cycle and a mitosis-related protein, proliferating cells	Tanaka A et al. (2007) *Mech. Ageing. Dev.* **128**, 303-310.
Nestin	Chemicon International, Temecula, CA, USA	Astrocytes, precursor cells	Chechneva O et al. (2005) *Neuroscience* **136**, 343-355.
DCX (doublecortin)	Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA	Early neuronal marker, neuroblasts	Plumpe T et al. (2006) *BMC Neurosci.* **7**, 77.

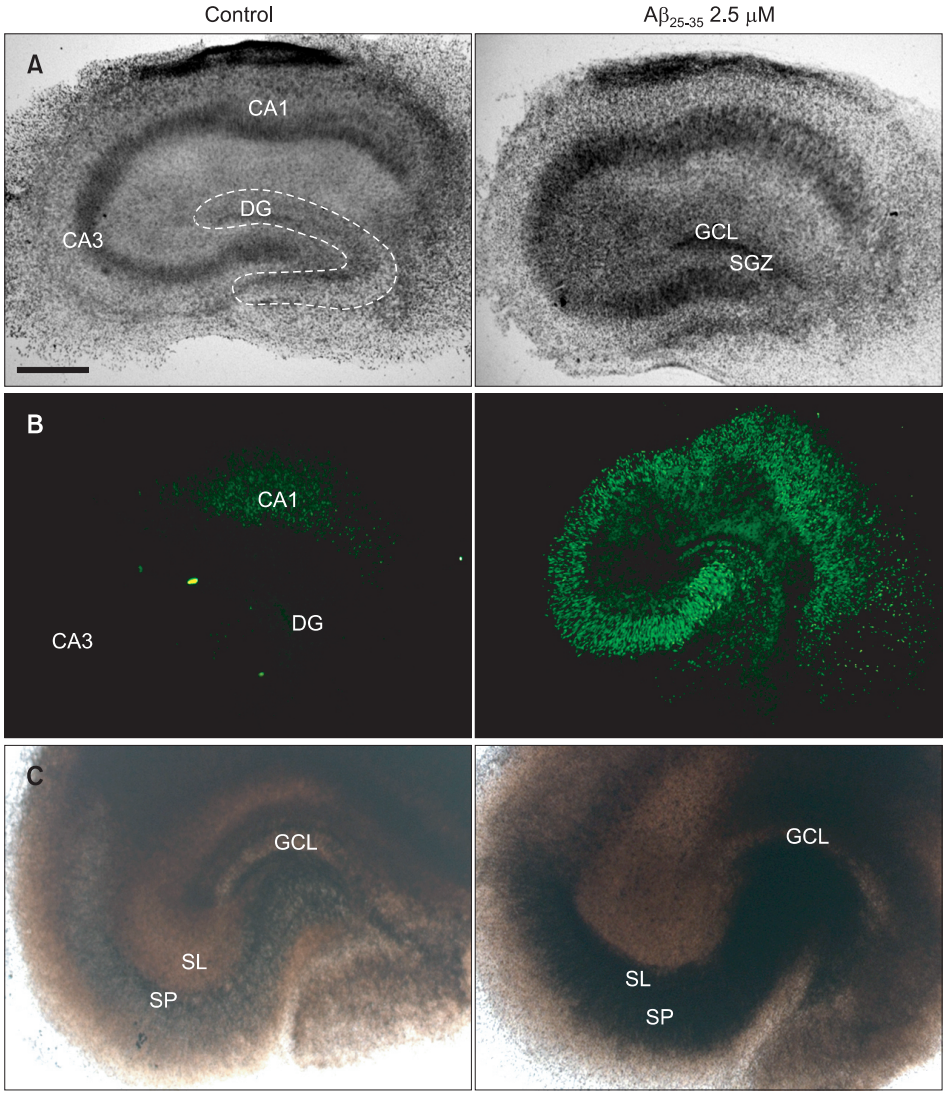

Fig. 1. Morphological changes in control and amyloid beta $(A\beta)_{25-35}$-treated organotypic hippocampal slice cultures. (A) 0.5% cresyl violet staining following treatment with $A\beta_{25-35}$ (2.5 μM). (B) Fluoro-Jade B-positive-cells were increased in the entire layer of pyramidal cells in $A\beta_{25-35}$ (2.5 μM)-treated slices. (C) Light microscopy images of Timm's staining in $A\beta_{25-35}$-treated organotypic hippocampal slice cultures. DG, dentate gyrus; GCL, granule cell layer; SGZ, subgranular zone; SL, stratum lucidum; SP, stratum pyramidale.

mouse or anti-rabbit secondary antibodies, conjugated to either fluorescein anti-mouse IgG (Vectoc Laboratories Inc., Burlingame, CA, USA), Alexa 488 goat anti-rabbit IgG (Life Technologies, Grand Island, NY, USA), or Alexa 555 goat anti-rabbit IgG (Life Technologies), were applied to the slices. The slices were then mounted with Elvanol mounting medium (Waterborne, New Orleans, LA, USA), and the images were obtained using confocal microscopy (Zeiss LSM 510; Zeiss, Jena, Germany) (Table 1).

Data Analysis

Data were analyzed by ANOVA using Statview version 5 softwares (SAS Institute, Cary, NC, USA). A Fisher's post hoc test was used to determine the significance. p-values<0.05 were considered to be statistically significant.

RESULTS

Evaluation of Neuronal Cell Damage in $A\beta_{25-35}$-treated Organotypic Hippocampal Slice Cultures

The hippocampal slice cultures were maintained *in vitro* for 21 days and neurotoxicity was induced by $A\beta_{25-35}$ (2.5 μM). In order to detect neuronal cell damage in the hippocampal slice cultures exposed to $A\beta_{25-35}$, cresyl violet staining and Fluoro-Jade B staining was performed. All pyramidal cell layers of the CA1, CA3, and DG regions were visualized with 0.5% cresyl violet staining, however, in comparison to control slices, neuronal damage was observed in the slices treated with $A\beta_{25-35}$ (2.5 μM) (Fig. 1A). In addition, dead and degenerating neurons were detected by Fluoro-Jade B staining in $A\beta_{25-35}$ (2.5 μM)-treated slices compared with control slices (Fig. 1B).

Changes in Mossy Fiber Terminals in $A\beta_{25-35}$-treated Organotypic Hippocampal Slice Cultures

Timm's staining for synaptic Zn^{2+} is especially intense in hippocampal mossy fibers (Huang, 1997). Following Timm's staining, brownish-black spots develop on mossy fiber terminals because these terminals contain a high concentration of Zn^{2+} vesicles. Brownish-black-stained spots in mossy fiber terminals of control slices were observed mainly in the stratum lucidum (SL), DG, and dentate hilus (Fig. 1C). In the slices treated with $A\beta_{25-35}$ (2.5 μM), the localization of these spots was expanded to the molecular layer and to various CA3 areas, including the SL and the stratum pyramidale (Fig. 1C).

Induction of Endogenous Neural Stem Cells in $A\beta_{25-35}$-treated Organotypic Hippocampal Slice Cultures

To evaluate the induction of endogenous neural stem cells, we employed immunofluorescence staining for the SGZ of the DG. In control slices, Ki-67 and Nestin, markers for newly proliferating cells and neural precursor cells, were distributed in the DG of the hippocampus. Following treatment with $A\beta_{25-35}$ (2.5 μM), the expression of both Ki-67 and Nestin was increased and some co-localized cells were found in the SGZ of DG. Ki-67 positive cells were significantly increased in 2.5 μM (from 6.63±0.43 to16±1.93) $A\beta_{25-35}$ treatment (Fig. 2). For the detection of proliferating cells, the hippocampal slices were incubated in BrdU for 24 h before fixation. BrdU-positive cells were distributed in both control and $A\beta_{25-35}$-treated hippocampal slices. In compared to the control, the BrdU positive cells were significantly increased in 2.5 μM (from 41±2.28 to 53.57±3.13). In order to detect neuronal precursor cells, immunofluorescence staining was performed

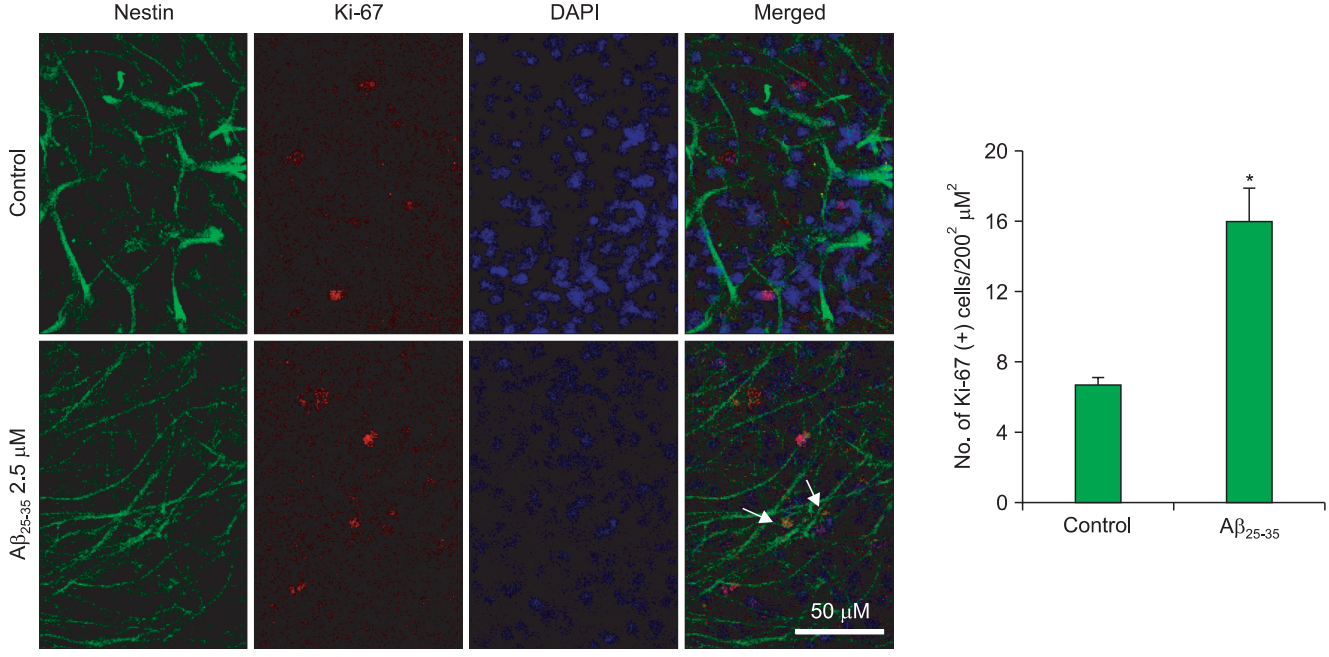

Fig. 2. Immunofluorescence staining of Ki-67 and Nestin in the SGZ of control and amyloid beta $(A\beta)_{25-35}$-treated organotypic hippocampal slice cultures. In control slices, Ki-67 and Nestin were distributed in the SGZ of DG. The expression of both Ki-67 and Nestin was increased and some co-localized cells were found by $A\beta_{25-35}$-treatment. The arrows showed co-localized Ki-67 and Nestin. Data are expressed as mean±SEM. *p<0.05 compared to control (n=5). SGZ, subgranular zone; DG, dentate gyrus; SEM, standard error of mean.

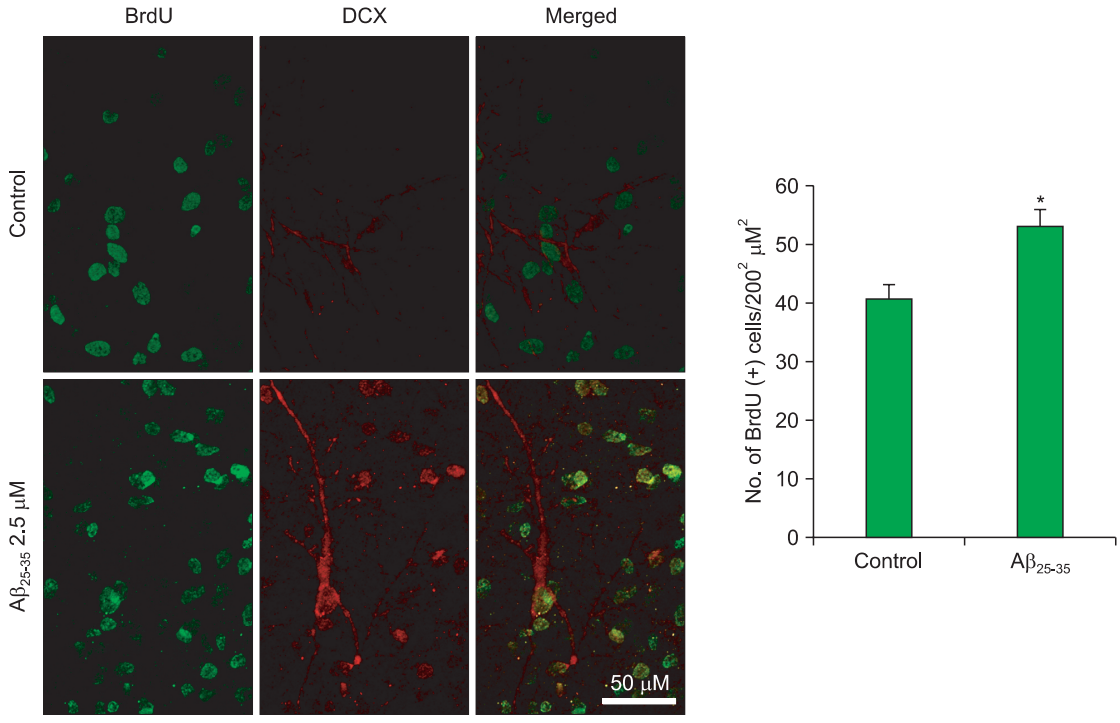

Fig. 3. Immunofluorescence staining of BrdU and DCX in the SGZ of control and $A\beta_{25-35}$-treated organotypic hippocampal slice cultures. In compared to the control, the BrdU positive cells were significantly increased in the SGZ of DG. Data are expressed as mean±SEM. *p<0.05 compared to control (n=7). BrdU, 5-Bromo-2′-deoxyuridine; DCX, doublecortin; SGZ, subgranular zone; Aβ, amyloid beta; DG, dentate gyrus; SEM, standard error of mean.

using a DCX antibody. After treatment with $A\beta_{25-35}$ (2.5 μM), DCX-expressing cells, with dendritic branching were detected by the presence of processes parallel to the granule cell layer (Fig. 3).

DISCUSSION

In the present study, we demonstrated that $A\beta_{25-35}$ induces reactive neurogenesis in the DG and the CA3 area of organotypic hippocampal slice cultures.

Neural stem cells, which can proliferate and differentiate, play a role in the repair of neuronal loss. Furthermore, they have the capacity to self-renew and differentiate into neurons and glia, and they can be cultured from the adult subventricular zone (SVZ) (Eriksson et al., 1998; Lie et al., 2004; Picard-Riera et al., 2004). Stimulation of both the proliferation and differentiation of endogenous neural progenitor cells and the transplantation of exogenous neural progenitor cells into the brain are potential therapeutic strategies in neurodegenerative disorders, such as AD (Mazur-Kolecka et al., 2006). In this study, we observed that treatment with $A\beta_{25-35}$ increased the number of neural stem cells and upregulated the differentiation of neural progenitor cells.

We have previously shown that $A\beta_{25-35}$ induces neurotoxicity in the CA1, CA3 and DG of hippocampal slice cultures using PI staining (Suh et al., 2008). In this study, we confirmed $A\beta_{25-35}$-induced neuronal cell death and degeneration in the all pyramidal cell layer of hippocampus using cresyl violet staining and Fluoro-Jade B staining.

It has been reported that mossy fibers, the axons of dentate granule cells, extend into the SL, a narrow region of the proximal apical dendrite of the CA3 pyramidal cells, where they form giant synapses that lead to the generation of new neuronal circuits (Tamura et al., 2006). Zn^{2+}, which binds Aβ with high affinity, is released at synaptic terminals during neurotransmission (Bush et al., 1994). Many studies have found alterations in Zn^{2+} levels in the AD brain (Faller, 2009), with most reporting an increase in its concentration (Religa et al., 2006). This collective evidence indicates that the regulation of Zn^{2+} is unbalanced in the AD brain and suggests a role for Zn^{2+} in AD pathogenesis (Deshpande et al., 2009; Bjorklund et al., 2012). Our study demonstrates that mossy fibers are abnormally distributed and extend outside of the CA3 area in $A\beta_{25-35}$-treated slices.

There is some evidence to suggest that the changes that occur in endogenous neural stem cells may vary with different models of AD. For example, it has been reported that the proliferation of endogenous neural stem cells is inhibited in the SVZ area of hippocampi in a rat AD model (Haughey et al., 2002), while on the other hand, it has also been suggested

that their capacity to repair is increased following exogenous application of $A\beta_{1-42}$ in a different model. In addition, BrdU labeled proliferating cells can be detected in dying neurons in CA1 (Kuan et al., 2004).

Although newly generated cells are commonly labeled with BrdU that is incorporated into their nuclei, it is not sufficient to prove the cellular and physiological features of endogenous neural stem cells. Therefore, we studied the presence of endogenous neural stem cells in hippocampal slice cultures using immunofluorescence staining for Ki-67, Nestin, and DCX. After $A\beta_{25-35}$ treament, BrdU- and Ki-67-positive cells in DG area were significantly increased and double positive cells for Ki-67/Nestin were observed, suggesting induction of neural stem cells. In addition, the intensity of glial fibrillary acidic protein (GFAP) was increased (data not shown), suggesting $A\beta_{25-35}$-induced gliosis. However, in this situation, we also found double positive cells for BrdU/DCX. This result is consistent with Perez-Gomez and Tasker (2012), reported that enhanced neurogenesis using BrdU/DCX after transient subfield-selective excitotoxic insult, and Bunk et al. (2010) reported NMDA-induced injury triggers neuroblast proliferation. This phenomenon may be a repair mechanism that is induced in response to $A\beta_{25-35}$-induced neurotoxicity, and $A\beta_{25-35}$ could induce neural stem cells not only for gliosis but also for neurogenesis.

CONCLUSIONS

These results demonstrate that Aβ-induced neuronal damage results in an increase in endogenous neural stem cells in rat hippocampal slice cultures. These findings suggest that proliferation and differentiation of neural stem cells following exposure to $A\beta_{25-35}$ may be a neuroprotective response that leads to the repair of degenerating neurons.

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REFERENCES

Bjorklund N L, Reese L C, Sadagoparamanujam V M, Ghirardi V, Woltjer R L, and Taglialatela G (2012) Absence of amyloid beta oligomers at the postsynapse and regulated synaptic Zn2+ in cognitively intact aged individuals with Alzheimer's disease neuropathology. *Mol. Neurodegener* **7**, 23.

Bunk E C, Konig H G, Bonner H P, Kirby B P, and Prehn J H (2010) NMDA-induced injury of mouse organotypic hippocampal slice cultures triggers delayed neuroblast proliferation in the dentate gyrus: an in vitro model for the study of neural precursor cell proliferation. *Brain Res.* **1359**, 22-32.

Bush A I, Pettingell W H, Multhaup G, d Paradis M, Vonsattel J P, Gusella J F, Beyreuther K, Masters C L, and Tanzi R E (1994) Rapid induction of Alzheimer A beta amyloid formation by zinc. *Science* **265**, 1464-1467.

Chechneva O, Dinkel K, Schrader D, and Reymann K G (2005) Identification and characterization of two neurogenic zones in interface organotypic hippocampal slice cultures. *Neuroscience* **136**, 343-355.

Chuang T T (2010) Neurogenesis in mouse models of Alzheimer's disease. *BBA* **1802**, 872-880.

Dehghani F, Hischebeth G T, Wirjatijasa F, Kohl A, Korf H W, and Hailer N P (2003) The immunosuppressant mycophenolate mofetil attenuates neuronal damage after excitotoxic injury in hippocampal slice cultures. *Eur. J. Neurosci.* **18**, 1061-1072.

Deshpande A, Kawai H, Metherate R, Glabe C G, and Busciglio J (2009) A role for synaptic zinc in activity-dependent Abeta oligomer formation and accumulation at excitatory synapses. *J. neurosci.* **29**, 4004-4015.

Eriksson P S, Perfilieva E, Bjork-Eriksson T, Alborn A M, Nordborg C, Peterson D A, and Gage F H (1998) Neurogenesis in the adult human hippocampus. *Nat. Med.* **4**, 1313-1317.

Faller P (2009) Copper and zinc binding to amyloid-beta: coordination, dynamics, aggregation, reactivity and metal-ion transfer. *Chembiochem: Eur. J. Chem. Biol.* **10**, 2837-2845.

Haughey N J, Liu D, Nath A, Borchard A C, and Mattson M P (2002) Disruption of neurogenesis in the subventricular zone of adult mice, and in human cortical neuronal precursor cells in culture, by amyloid beta-peptide: implications for the pathogenesis of Alzheimer's disease. *Neuromolecular Med.* **1**, 125-135.

Haydar T F, Bambrick L L, Krueger B K, and Rakic P (1999) Organotypic slice cultures for analysis of proliferation, cell death, and migration in the embryonic neocortex. *Brain Res.* **4**, 425-437.

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.

Huang E P (1997) Metal ions and synaptic transmission: think zinc. *Proc. Natl. Acad. Sci. U S A* **94**, 13386-13387.

Kuan C Y, Schloemer A J, Lu A, Burns K A, Weng W L, Williams M T, Strauss K I, Vorhees C V, Flavell R A, Davis R J, Sharp F R, and Rakic P (2004) Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. *J. Neurosci.* **24**, 10763-10772.

Lie D C, Song H, Colamarino S A, Ming G L, and Gage F H (2004) Neurogenesis in the adult brain: new strategies for central nervous system diseases. *Ann. Rev. Pharm. Toxicol.* **44**, 399-421.

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.

Lopez-Toledano M A and Shelanski M L (2007) Increased neurogenesis in young transgenic mice overexpressing human APP(Sw, Ind). *J.*

Alzheimer's Dis. **12**, 229-240.

Mattson M P (2000) Apoptosis in neurodegenerative disorders. *Nat. Rev. Mol. Cell Bio.* **1**, 120-129.

Mazur-Kolecka B, Golabek A, Nowicki K, Flory M, and Frackowiak J (2006) Amyloid-beta impairs development of neuronal progenitor cells by oxidative mechanisms. *Neurobiol. Aging* **27**, 1181-1192.

Namba T, Mochizuki H, Onodera M, Namiki H, and Seki T (2007) Postnatal neurogenesis in hippocampal slice cultures: early in vitro labeling of neural precursor cells leads to efficient neuronal production. *J. Neurosci. Res.* **85**, 1704-1712.

Perez-Gomez A and Tasker R A (2012) Enhanced neurogenesis in organotypic cultures of rat hippocampus after transient subfield-selective excitotoxic insult induced by domoic acid. *Neuroscience* **208**, 97-108.

Picard-Riera N, Nait-Oumesmar B, and Baron-Van Evercooren A (2004) Endogenous adult neural stem cells: limits and potential to repair the injured central nervous system. *J. Neurosci. Res.* **76**, 223-231.

Plumpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, Romer B, Rodriguez G R, Kronenberg G, and Kempermann G (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci.* **7**, 77.

Raineteau O, Rietschin L, Gradwohl G, Guillemot F, and Gahwiler BH (2004) Neurogenesis in hippocampal slice cultures. *Mol. Cell. Neurosci.* **26**, 241-250.

Religa D, Strozyk D, Cherny R A, Volitakis I, Haroutunian V, Winblad B, Naslund J, and Bush A I (2006) Elevated cortical zinc in Alzheimer disease. *Neurology* **67**, 69-75.

Rodriguez J J, Jones V C, and Verkhratsky A (2009) Impaired cell proliferation in the subventricular zone in an Alzheimer's disease model. *Neuroreport* **20**, 907-912.

Rola R, Raber J, Rizk A, Otsuka S, VandenBerg S R, Morhardt D R, and Fike J R (2004) Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. *Exp. Neurol.* **188**, 316-330.

Sadgrove M P, Laskowski A, and Gray W P (2006) Examination of granule layer cell count, cell density, and single-pulse BrdU incorporation in rat organotypic hippocampal slice cultures with respect to culture medium, septotemporal position, and time in vitro. *J. Comp. Neurol.* **497**, 397-415.

Scheffler B, Schmandt T, Schroder W, Steinfarz B, Husseini L, Wellmer J, Seifert G, Karram K, Beck H, Blumcke I, Wiestler O D, Steinhauser C, and Brustle O (2003) Functional network integration of embryonic stem cell-derived astrocytes in hippocampal slice cultures. *Development (Cambridge, England)* **130**, 5533-5541.

Schaffer D V and Gage F H (2004) Neurogenesis and neuroadaptation. *Neuromolecular Med.* **5**, 1-9.

Suh E C, Jung Y J, Kim Y A, Park E M, and Lee K E (2008) A beta 25-35 induces presynaptic changes in organotypic hippocampal slice cultures. *Neurotoxicology* **29**, 691-699.

Tamura M, Koyama R, Ikegaya Y, Matsuki N, and Yamada M K (2006) K252a, an inhibitor of Trk, disturbs pathfinding of hippocampal mossy fibers. *Neuroreport* **17**, 481-486.

Tanaka A, Watanabe Y, Kato H, and Araki T (2007) Immunohistochemical changes related to ageing in the mouse hippocampus and subventricular zone. *Mech. Ageing Dev.* **128**, 303-310.

Tanvig M, Blaabjerg M, Andersen R K, Villa A, Rosager A M, Poulsen F R, Martinez-Serrano A, Zimmer J, and Meyer M (2009) A brain slice culture model for studies of endogenous and exogenous precursor cell migration in the rostral migratory stream. *Brain Res.* **1295**, 1-12.

Wang J M, Irwin R W, Liu L, Chen S, and Brinton R D (2007) Regeneration in a degenerating brain: potential of allopregnanolone as a neuroregenerative agent. *Curr. Alzheimer Res.* **4**, 510-517.

Wang S H, Zhang Z J, Guo Y J, Teng G J, and Chen B A (2008) Hippocampal neurogenesis and behavioural studies on adult ischemic rat response to chronic mild stress. *Behav. Brain Res.* **189**, 9-16.

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.

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.