A study on the attenuating effects of neuregulin-1 on cognitive function impairments and its action mechanisms in an Alzheimer's disease animal model

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의학박사 학위논문

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뉴레글린-1 의 알츠하이머병 동물 모델에서의 인지기능 개선 효과 및 그 기전에 관한 연구

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류 정 화

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by

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ABSTRACT

Introduction: Alzheimer's disease (AD), the most common form of dementia, is a chronic neurodegenerative disease causing progressive impairment of memory and other cognitive functions. Pathologically, AD brain is characterized by two types of lesion: senile or neuritic plaques and neurofi brillary tangles. It has been proposed that AD is a synaptic failure. Loss of synapses at a fine structural level as well as reduction in synaptic markers have been well documented in early and late stages of AD and have been shown to correlate well with the extent of cognitive deficits. The neuregulin (NRG) family of epidermal growth factor-related proteins is composed of a wide variety of soluble and membrane-bound proteins that mediate their effects via the tyrosine kinase receptors ErbB2 - ErbB4. In the nervous system, the functions of NRG1 are essential for peripheral myelination, the establishment and maintenance of the neuromuscular and sensorimotor systems and the plasticity of cortical neuronal circuits. Heterozygous NRG1-KO mice show impairments in prepulse inhibition and working memory.

Methods & Results: In this study, I demonstrate that an intracerebroventricular infusion of NRG1 attenuates the cognitive function impairments in 13-month-old Tg2576 mice, an animal model of AD. NRG1 was found to rescue the reduction in the number of dendritic spines density in the brain tissues of Tg2576 mice, compared to vehicle-infused mice based on Golgi-Cox staining. This result was also corroborated *in vitro* in which NRG1 attenuates the

oligomeric amyloid beta peptide$_{1-42}$ ($A\beta_{1-42}$)-induced reduction in dendritic spine density of rat primary hippocampal neuron cultures. Furthermore, NRG1 was demonstrated to alleviate the reduction in neural differentiation induced by oligomeric $A\beta_{1-42}$ in mouse fetal neural stem cells.

Conclusions: Taken together, these results suggest that NRG1 has a therapeutic potential for AD by alleviating the reductions in dendritic spine density and neurogenesis in AD brains.

Keywords: Alzheimer's disease, Neuregulin-1, Cognitive impairment, Neurogenesis, Synaptic plasticity

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LIST OF ABBREVIATIONS

AD: Alzheimer’s disease

ADAM: a disintergrin and metallopeptidase

APP: amyloid precursor protein

ARIA: acetylcholine receptor-inducing activity

BACE1: β-site-APP cleaving enzyme 1

bFGF: basic fibroblast growth factor

CNS: central nervous system

DAPI: 4’.6-diamidino-2-phenylindole

EGF: epidermal growth factor

ErbB: epidermal growth factor receptor

GEF: guanine exchange factor

GFAP: glial fibrillary acidic protein

GGF: glial growth factor

HRG: heregulin

IRES: internal ribosome entry site

LTP: long-term potentiation

MAP2: microtubule associated protein-2

NDF: neural differentiation factor

NRG: neuregulin

oAβ_{1-42}: oligomeric amyloid beta peptide$_{1-42}$

PSD95: postsynaptic density 95

RNAi; RNA interference

SMDF: sensory and motor neuron-derived factor

SNPs: single nucleotide polymorphisms

Tg: transgenic

TNF-α: tumor necrosis factor-alpha

WT: wild-type

INTRODUCTION

Alzheimer's disease (AD), first described by the German neuropathologist Alois Alzheimer as Dementia Praecox in 1907, is an age-related neurodegenerative disease characterized by progressive loss of memory and deterioration of cognitive functions. Individuals with the disorder usually experience difficulties in learning, performance speed, recall accuracy and/or problem solving. AD is characterized by two neuropathological hallmarks, neuritic plaques and neurofibrillary tangles. AD primarily targets synapses, and synaptic loss and dysfunction have been reported to be well correlated with cognitive dysfunction in AD [1-3]. Immunoreactivites against synaptophysin, a presynaptic marker protein and PSD-95, a postsynaptic marker protein are reduced in the brains of AD patients and mouse models of AD [4, 5]. Spine density reduction was also reported in AD brains [6, 7]. In addition, it has been reported that oligomeric amyloid beta peptide (Aβ) disrupts neurogenesis in SVZ and hippocampus in mouse models of AD [8].

Neuregulins (NRGs) comprise a large family of widely expressed epidermal growth factor (EGF)-like proteins that have been implicated in neural development and brain activity homeostasis. NRG1 was the first member of the family to be discovered at 1992, for very different biological functions, including activation of ErbB family, stimulation of Schwann cell growth, and induction of acetylcholine receptor expression [9, 10]. Since then, five additional NRG genes (NRG2, NRG3, NRG4, NRG5, and NRG6) have been identified. Each NRG gene gives rise to multiple splice isoforms (>30 for

NRG1 and >15 for NRG3, for example) [10, 11]. Immature NRGs are transmembrane proteins, which release (upon proteolytic processing) soluble N-terminal moieties that contain the EGF-like signaling domain. NRGs and related EGF domain-containing proteins interact with and activate receptor tyrosine kinases of the ErbB family, each of which initiates intracellular signaling pathways in a specific way, including noncanonical mechanisms. NRG/ErbB signaling has been implicated in neural development including circuitry generation, axon ensheathment, neurotransmission, and synaptic plasticity. [12, 13]. NRGs and their receptors, ErbBs, have been identified as susceptibility genes for diseases such as schizophrenia and bipolar disorder. Recent studies have revealed complex NRG/ErbB signaling networks that regulate the assembly of neural circuitry, myelination, neurotransmission, and synaptic plasticity. Evidence indicates there is an optimal level of NRG/ErbB signaling in the brain and deviation from it impairs brain functions. Compared to NRG1, little is yet known regarding the biological functions of the NRG2, 3, 4, 5 and 6 proteins.

NRG1, which includes at least 15 isoforms via alternative splicing [14, 15], have been demonstrated to exhibit diverse functions in the development of the nervous system and play multiple essential roles in vertebrate embryogenesis, including cardiac development, Schwann cell and oligodendrocyte differentiation and the neuromuscular synapse formation [16, 17].

NRG1 is expressed in the brain at synaptic regions [16, 18, 19], and its signaling can control synapse maturation and plasticity [20]. Moreover, in the hippocampus, NRG1 mRNA is highly expressed in the CA3 area [19] .

The three structural characteristics to importantly differentiate isoforms with respect to *in vivo* functions and cell biological properties are the type of EGF-like domain (α or β), the N-terminal sequence (type I, II, or III), and whether the isoform is initially synthesized as a transmembrane or nonmembrane protein. The EGF-like domain contained in all bioactive NRG isoforms is alone sufficient for activation of ErbBs [15]. The names first used in the literature to refer to various NRG isoforms—acetylcholine receptor-inducing activity (ARIA) [21], glial growth factor (GGF) [22, 23], heregulin (HRG) [24], neural differentiation factor (NDF) [25, 26], and sensory and motor neuron-derived factor (SMDF) [27].

Various functions of NRG1 were revealed using knockout and other strategies; Schizophrenia [28, 29], multiple sclerosis [30], promotion of neural regeneration/proliferation of olfactory ensheathning glia [31, 32], traumatic brain injury [33], Trastuzumab cardiotoxicity [34].

Homozygous NRG1 knockout (NRG1-KO) mice do not survive beyond the embryonic stage of development [35], but heterozygous NRG1-KO mice exhibit impairments in prepulse inhibition and working memory [28, 36]. NRG1 activity is highly dependent on cleavage by β-site-APP cleaving enzyme 1 (BACE1). BACE1 also participates in the cleavage of amyloid precursor protein (APP) leading to the generation of Aβ which is central to the pathogenesis of AD. Furthermore, NRG1 is processed by γ-secretase after cleavage by BACE1.

BACE1 KO mice have been reported to share distinctly equivalent features to heterozygous NRG1-KO mice [37]. Consequently it can be hypothesized that

NRG1 is important for cognition and learning and memory formation via the modulation of synaptic plasticity, neurite extension and neuronal survival and that NRG1 is important molecule in the pathogenesis of AD.

Despite the rapidly expanding knowledge regarding the functions of NRG1 in the developing brain, little is currently known regarding the influence of NRG1 on the mature nervous system as well as in neurodegenerative diseases such as AD. AD is neuropathologically characterized by the presence of neuritic plaques principally composed of Aβ and neurofibrillary tangles [38]. The pathogenesis of AD could be explained by a loss in neural plasticity [39, 40] that may adversely affect dendritic arborizations, synaptic remodeling, long-term potentiation (LTP), axonal sprouting, synaptogenesis, and neurogenesis.

In this study, I investigated the effects of NRG1 and its underlying mechanism in *in vitro* and *in vivo* experimental models of AD.

MATERIALS AND METHODS

1. Reagents and antibodies

Recombinant human NRG1/Heregulin-β2 (rHuNRG1) was purchased from PROSPEC (East Brunswick, NJ, USA). AG1478, an inhibitor of ErbB4, was purchased from Calbiochem (Darmstadt, Germany) and dissolved in dimethylsulfoxide (DMSO; Duchefa biochemi, Haarlem, Netherlands). The $A\beta_{1-42}$ was obtained from Sigma Chemical Company (St. Louis, MO, USA). A rabbit anti-glial fibrillary acidic protein (anti-GFAP) polyclonal antibody was purchased from Dakocytomation (Glostrup, Denmark). A mouse anti-microtubule associated protein-2 (MAP2), clone AP 20 monoclonal antibody was purchased from Millipore (Billerica, MA, USA). A mouse anti-postsynaptic density 95 (PSD95) clone, 7E3-1B8 monoclonal antibody was purchased from Thermo (Meridian Rd, Rockford, IL USA); a mouse anti-tau monoclonal antibody was, from Cell Signaling Technology (Danvers, MA, USA). The fluorescent secondary antibodies, Alexa 488-conjugated goat α-mouse IgG and Alexa 555-conjugated goat α-rabbit IgG were obtained from Invitrogen (Carlsbad, CA, USA).

2. Transgenic mice

All *in vivo* experiments were performed in accordance with 'the Guidelines for Animal Experiments set forth by the Ethics Committee of Seoul National University'. Tg2576 mice were obtained from Taconic Farms (Germantown,

NY, USA) and were bred by mating male with C57B16/SJL F1 female mice, as recommended by the suppliers and as described by others [41]. Comparisons were performed between heterozygous transgenic (Tg2576) mice to age-matched transgene-negative littermates (wild type; WT). Mice were separated into 4 groups (3~6 mice per group).

3. Intracerebroventricular infusion of NRG1 using an osmotic pump

Alzet Model 1004 Micro-Osmotic Pumps (DURECT, Cupertino, CA, USA), with a capacity of 100 μL and an injection rate of 0.11 μL/ h for 4 weeks were used in this study. The osmotic pumps were filled with NRG1 peptide (28 ng/ kg/ in mouse) or phosphate buffered saline (PBS) according to the manufacturer's instructions. The pre-filled pumps were then placed in PBS at 37 °C for 24 h. Mice were fitted with a stainless steel cannula which was supplied with the Alzet Brain Infusion kit (DURECT), via insertion into the right lateral ventricle of the brain according to stereotaxic coordinates. The osmotic pumps were subsequently implanted subcutaneously into the back and connected to the fitted cannula. All surgical procedures were performed under anaesthesia via intramuscular injection of Rompun (17.5 mg/ kg) and Zoletil (12.5 mg/ kg). All mice were housed individually during the post-surgical observation period.

4. Morris water maze task

The Morris water maze task was performed 4 weeks after intracerebroventricular infusion of vehicle or NRG1 using an osmotic pump. The experimental apparatus, which is a circular water tank (diameter = 140 cm, height = 45 cm), was filled with opaque water produced by adding dry milk powder to water at room temperature (21-23 ℃). Animals were required to find a submerged platform (diameter =12 cm, height = 35 cm) in the pool using spatial cues. 3 training trials per day were conducted for 4 consecutive days, in which the initial placement of the mice into the maze was rotated per trial for each group. For each training session, the latency to escape to the hidden platform was recorded. 48 h after the final trial session, a single probe trial was conducted. The escape platform was removed and each mouse was allowed to swim for 60 s in the maze.

5. Tissue preparation

Mice were anesthetized via intramuscular injection of Rompun (17.5 mg/ kg) and Zoletil (12.5 mg/ kg) and sacrificed after behavioral testing. The mice were immediately intracardially perfused with PBS containing heparin. After perfusion, each brain was extracted and fixed in 4 % paraformaldehyde solution for 24 h at 4℃ and incubated in 30 % sucrose solution for 48 h at 4℃ for immunohistochemical analysis. Sequential 25 μm coronal were generated using a cryostat (Cryotome, Thermo Electron Cooperation, MA, USA) and stored at 4℃.

6. Golgi-Cox staining

Golgi-Cox staining was performed using the FD Rapid GolgiStain Kit (FD Neuro Technologies, MD, USA), as previously described [42, 43]. Briefly, animals were deeply anesthetized, and the brains were removed from the skulls and rinsed in double distilled water. The brains were immersed in impregnation solution, and stored at room temperature for 2 weeks in the dark. The impregnation solution was replaced the following day, and the brains were transferred to Solution C. Solution C was replaced the next day and the brains were stored at 4°C for 3 days in the dark. Brain sections (120 μm thickness) were generated using a Cryotome (Thermo Electron Cooperation, MA, USA) with the chamber temperature set at -22°C. Each section was mounted on saline-coated microscope slides using Solution C. After the absorption of excess solution, the sections were allowed to dry naturally at room temperature. The dried sections were processed according to the manufacturer's instructions. Briefly, dendrites within the CA1 subregion of the hippocampus were imaged using a 100 × objective on a Olympus BX-51 microscope with a Leica DFC 280 digital camera. Dendritic spines were detected along CA1 secondary dendrites starting from their point of origin from the primary dendrite.

7. Immunohistochemistry

Brain sections were incubated in 0.3 % Triton X-100 for 10 min for permeabilization, blocked with 10 % normal goat serum The sections were incubated with primary antibodies overnight, followed by incubation with appropriate fluorescence-associated secondary antibodies and 1 μM 4’.6-

diamidino-2-phenylindole (DAPI) for 1 h at room temperature. After three washes in permeabilization buffer and a wash in PBS, sections were mounted on microscope slides in mounting medium (DAKO, CA). Confocal microscopic observation was performed using LSM 510 (Carl Zeiss, Jena, Germany).

8. Primary hippocampal neuron culture

The hippocampus was removed from Sprague-Dawley rat embryos (E18). Cells were dissociated using 0.25 % trypsin and plated onto coverslips coated with poly-L-lysine. Cells were transfected with 3 μg of IRES-mGFP vector using CalPhos Mammalian Transfection Kit (Clontech Laboratories, CA, USA. The neurons were grown in Neurobasal medium (Gibco, CA, USA) supplemented with B27 (Gibco), 2 mM GlutaMAX-I supplement (Gibco) and 100 μg/ ml penicillin/streptomycin (Gibco, CA, USA) at 37 ºC in a humidified environment with 5% CO_2.

9. Oligomeric Aβ preparation

I used synthetic $A\beta_{1-42}$ peptides with > 95 % purity based on RP-HPLC chromatography (American Peptide Company, Sunnyvale, CA, USA). Briefly, the $A\beta_{1-42}$ was dissolved to 1 mM in 100 % hexafluoroisopropanol (Sigma Chemical Company, St Louis, MO, USA). Next, the hexafluoroisopropanol was removed under vacuum, and the peptide was stored at −20°C. Then, for oligomeric conditions, F-12 (without phenol red) culture medium was added to

dissolve the peptide to a final concentration of 100 μM, and the peptide was incubated at 4°C for 24 h.

10. Dendritic spine density analysis

Primary hippocampal neuron cultures (days per *in vitro* 12, DIV 12) were transfected with IRES-mGFP vector. The number of dendritic spines was evaluated at DIV 17. Fluorescent images were acquired with an LSM 510 confocal microscope (Carl Zeiss, Jena, Germany), using the same settings for all of the samples. The spines were counted within the 50- to 100- μm segments on the secondary dendrites that extended at least 40-80 μm beyond the cell body (soma).

11. Quantification of neurite outgrowth

For assessing neurite outgrowth, the cultures were fixed with 4 % paraformaldehyde in PBS (pH 7.4) for 20 min at room temperature. Fixed cells were then incubated in blocking buffer with 10 % normal horse serum and PBS for 1 h then rinsed three times with PBS. Neurons were labelled by indirect immunofluorescence using a primary antibody to tau and an Alexa Fluor 488 conjugated secondary antibody. After immunostaining, confocal microscopy was performed in a Zeiss LSM 510 confocal microscope. Neurite outgrowth was quantified using a software ZEN 2012 (Carl Zeiss)

12. Immunocytochemistry

Cells were fixed with 4 % paraformaldehyde for 30 min at room temperature. Then the cells were washed with PBS, followed by permeabilization with PBS containing 0.05 % Tween 20. Next, the cells were blocked with blocking buffer (3 % normal goat serum, 2 % BSA and 3 % Triton-X 100 in PBS) for 1 h at room temperature. After washing, the cells were incubated in PBS containing appropriate primary antibodies for 2 h at room temperature. After the incubation, the cells were washed with PBS. Then the cells were incubated in PBS containing Alexa Flour 488- or 546- conjugates secondary antibodies, and 1 μM DAPI for 1h at room temperature. Cells were then observed under a confocal microscope (Zeiss LSM 510)

13. Fetal neural stem cell culture

Fetal neural stem cell culture was performed as previously described [44]. For fetal neural stem cell culture, cerebral cortex and hippocampus were isolated at embryonic day 13 from C57BL/6 mice. Cells were isolated via mechanical dissociation in minimum essential medium (MEM) supplemented with 1 % penicillin/streptomysin (pH 7.2), and 10^7 cells were cultured in a T-75 flask. Neurospheres derived from fetal neural stem cells were generated as previously described [45]. For immunocytochemistry analysis, I dissociated the neurospheres and subcultured the fetal neural stem cells (5×10^4 cells/well) in 24-well plates that contained 12 mm glass coverslips (Marienfeld, Lauda-Konigshofen, Germany) pre-coated with laminin (Roche, Mannheim, Germany) at a concentration of 20 μg/ ml in MEM with 5 μM HEPES and

penicillin/streptomycin. The cells were incubated in the differentiation medium without growth factors (EGF and bFGF) for 5 days and were analyzed via immunocytochemistry

14. Statistical analysis

Data are expressed as the means ± SEM. One-way ANOVA (SPSS, IL, USA) was performed to determine statistical significance. The results were considered to be statistically significant if $p < 0.05$.

RESULTS

NRG1 attenuates the impairments in learning and memory in 13-month-old Tg2576 mice

Tg2576 mouse model of AD overexpresses the 695-amino acid isoform of human APP containing a Lys 670 – Asn, Met 671 – Leu mutation [46]. As Tg2576 mice age, amyloid plagues with Aβ appear that are similar to those seen in AD. In this mice, rapid increases in a 5 -fold increase in $A\beta_{1-40}$ and a 14 -fold increase in $A\beta_{1-42/43}$ staring at ~ 6 months and amyloid plaques beginning at 9-12 months [47]. Also Tg2576 mice show behavioral deficits as assessed by Y maze, T maze and Morris water maze testing with age [46, 47].

First, I tested whether NRG1 ameliorates the learning and memory deficits observed in Tg2576 mice, an animal model of AD. I infused vehicle or NRG1 into the lateral ventricle of 12 -month-old WT or Tg2576 mice via an osmotic pump inserted using a stereotaxic apparatus for 4 weeks. After NRG1 or PBS infusion, I performed Morris water maze test and a single probe trial. As I show the schematic diagram of the experimental procedure is provided in Fig. 1A.

4 weeks after the infusion, I assayed the spatial learning and memory capabilities using Morris water maze test in the WT-vehicle, WT-NRG1, Tg2576-vehicle and Tg2576-NRG1 infused mice. The group of NRG1-infused 13 -month-old Tg2576 mice exhibited shorter latency times (day 3 : 43.83 ± 4.98, day 4 : 40.41 ± 5.92, n=4) than the group of vehicle-infused Tg2576 mice(day 3 : 58.33 ± 1.67, day 4 : 55.11 ± 4.89, n=3) of the same age on days 3 and 4 of these learning sessions (Fig. 1B). On day 6, I performed a probe test

in which the platform was removed, and I recorded the average latency in zone 4, where the platform had been placed during the training period. NRG1-infused WT and Tg2576 mice remained in zone 4 significantly longer than the other zones (zones 1, 2 and 3) (a one-way ANOVA, $^{**}p < 0.01$, $^{***}p < 0.001$, respectively, Fig 1C). However, no significant differences were detected for the remaining two groups of mice with respect to the times spent in each zone (Fig. 1C).

NRG1 rescues the reduction in dendritic spine density in Tg2576 mice as assessed with Golgi-Cox staining

AD is a disease that is caused by synaptic failure [48]. In Tg2576 mice, no statistically significant difference in spine density was found between 2 -month-old Tg2576 and WT mice. A significant reduction in spine density was found in 4 -month-old Tg2576 compared with WT mice. In 12 -month-old Tg2576 mice, the reduction was also significant [49].

I evaluated the dendritic spines via Golgi-Cox staining in WT-PBS, WT-NRG1, Tg2576-PBS and Tg2576-NRG1-infused mice after performing the Morris water maze test. As shown in Fig. 2A and 2B, Tg2576 mice exhibited the reduced dendritic spine density in the CA1 region, compared to WT mice. It was found that NRG1 infusion in Tg2576 mice alleviated the reduction in dendritic spine density in the CA1 region (the bottom panel, Fig. 2B and 2C). Tg2576-PBS-infused mice significantly reduced the number of dendritic spines per 10 μm of dendrites (17.06 ± 7.09, n= 11, $^{***}p < 0.001$), compared to WT-PBS-infused mice (25.03 ± 1.04, n= 23). Tg2576-NRG1-infused mice

alleviated the reduction in the number of dendritic spines (23.24 ± 0.44, n= 16, ###$p < 0.001$) compared to Tg2576-PBS-infused mice (17.06 ± 7.09, n= 11).

NRG1 alleviates the reduction in PSD95 immunoreactivity in Tg2576 mice.

PSD95 is a postsynaptic membrane marker which represents synapse formation. I investigated the immunoreactivity against PSD95 via immunohistochemistry in the WT-PBS, WT-NRG1, Tg2576-PBS and Tg2576-NRG1 infused mice. NRG1 infusion alleviated the decrease in PSD95 expression in the hippocampal region in Tg2576 mice (Fig. 3).

NRG1 rescues the reduction in dendritic spine density induced by oligomeric $A\beta_{1-42}$ in rat primary hippocampal neuron cultures

The majority of the excitatory synapses in the mammalian central nervous system (CNS) are formed on dendritic spines, and spine morphology and distribution are critical for synaptic transmission, synaptic integration and plasticity [50, 51]. The presence of soluble Aβ oligomers in brain is highly correlated with synaptic dysfunction in AD [48]. Accumulation of Aβ peptide, derived via APP proteolysis, is responsible for age-related memory decline in Tg2576 model [47, 52, 53]. Oligomeric $A\beta_{1-42}$ treatment has been reported to induce a reduction in dendritic spine density. I confirmed that the treatment of rat primary hippocampal neuron cultures with 250 nM oligomeric $A\beta_{1-42}$ for 4 days induced a significant reduction in dendritic spine density (8.45 ± 0.35 / 10 μm, n=16, $p< 0.001$), compared to the vehicle-treated neurons (11.55 ± 0.39 / 10 μm, n=18).

To examine the effects of NRG1 at the synapse, primary hippocampal neurons were transfected with IRES-mGFP vector at DIV 12 and treated with 10 nM NRG1 at DIV 14 and I analyzed the number of dendritic spines at DIV 17 (Fig. 4A). The treatment with 10 nM NRG1 for 3 days significantly upregulated the number of dendritic spines by 23.6 % (14.27 ± 0.35 / 10 μm, n= 19, $p < 0.001$), compared to the vehicle-treated neurons (11.55 ± 0.39 / 10 μm, n= 18). This result is consistent with a previous report demonstrating that NRG1 heterozygous mice exhibit decreased spine densities within proximal regions of apical dendrites of hippocampal pyramidal neurons compared to WT [54]. The treatment with 10 nM NRG1 1 day after the treatment of oligomeric $A\beta_{1-42}$ for 3 days (from DIV 14 to DIV 17) rescued the reduction in dendritic spine density induced by the treatment with oligomeric $A\beta_{1-42}$ (13.97 ± 0.37 / 10 μm, n= 19, $p < 0.001$), compared to the oligomeric $A\beta_{1-42}$ plus vehicle treated neurons (8.45 ± 0.35 / 10 μm, n= 16) (Figs. 4A and 4B).

Ablation of ErbB4 from excitatory neurons leads to reduced dendritic spine density in mouse prefrontal cortex [55]. To confirm the effects of NRG1 via ErbB4 at the synapse, primary hippocampal neurons were treatment with AG1478, ErbB4 inhibitor at DIV 14 and treated with 10 nM NRG1 at DIV 14 and I analyzed the number of dendritic spines at DIV 17 (Fig. 4A). The treatment with 250 nM AG1478 at DIV 14 reduced the dendritic spine density (9.58 ± 0.38 / 10 μm, n= 19, $p < 0.001$), compared to the vehicle-treated neurons (11.55 ± 0.39 / 10 μm, n= 18). However, the co-treatment with AG1478 and NRG1 rescue the reduction in dendritic spine density (14.58 ±

0.39 / 10 μm, n= 19, $p < 0.001$), indicating that this action of NRG1 is independent with ErbB4. The co-treatment with 250 nM oligomeric $A\beta_{1-42}$ and 250 nM AG1478 at DIV 14 reduced the dendritic spine density (9.07 ± 0.27 / 10 μm, n= 13, $p < 0.001$), compared to the vehicle-treated neurons (11.55 ± 0.39 / 10 μm, n= 18). The treatment with 10 nM NRG1 1 day after the treatment of 250 nM oligomeric $A\beta_{1-42}$ and 250 nM AG1478 for 3 days (from DIV 14 to DIV 17) rescued the reduction in dendritic spine density induced by the treatment with oligomeric $A\beta_{1-42}$ (15.74 ± 0.34 / 10 μm, n= 19). This results also indicate that this action of NRG1 is independent with ErbB4.

NRG1 has attenuating effect against oligomeric $A\beta_{1-42}$ -induced reduction of neurite outgrowth

To further determine whether NRG1 affects the reduction in neurite outgrowth induced by oligomeric $A\beta_{1-42}$, I tested the effects of NRG1 in rat primary hippocampal neuron cultures under the same treatment conditions used while evaluating dendritic spine density. As shown in Figs 5A and 5B, neurite outgrowth in the neurons exposed to oligomeric $A\beta_{1-42}$ (250 nM) for 4 days was reduced significantly (253.73 ± 23.72 μm, n= 6, $p < 0.01$), compared to the vehicle-treated controls (363.41 ± 10.00 μm, n= 6) by 41.3 %. The co-treatment with 10 nM NRG1 for 3 days restored neurons from oligomeric $A\beta_{1-42}$ -induced reduction of neurite outgrowth to 350.72 ± 20.00 μm ($p < 0.01$, n= 6; Fig. 5B). These results indicate that NRG1 has a significant attenuating effect against oligomeric $A\beta_{1-42}$ -induced reduction of neurite outgrowth.

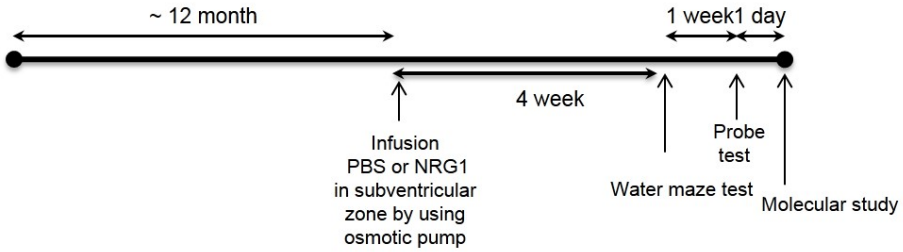
NRG1 rescues the impairment in neural differentiation induced by oligomeric $A\beta_{1-42}$ in fetal neural stem cells

Next, we examined whether NRG1 affects the impaired neural differentiation induced by treatment with oligomeric $A\beta_{1-42}$ in fetal neural stem cells. Prior to investigation of the effects of NRG1 and $A\beta_{1-42}$ in the cells, immunofluorescence was performed using a specific antibody against MAP2, which labels neurons and GFAP for astroglial cells to confirm the characteristics of stem cells and to identify neurons and astrocytes differentially.

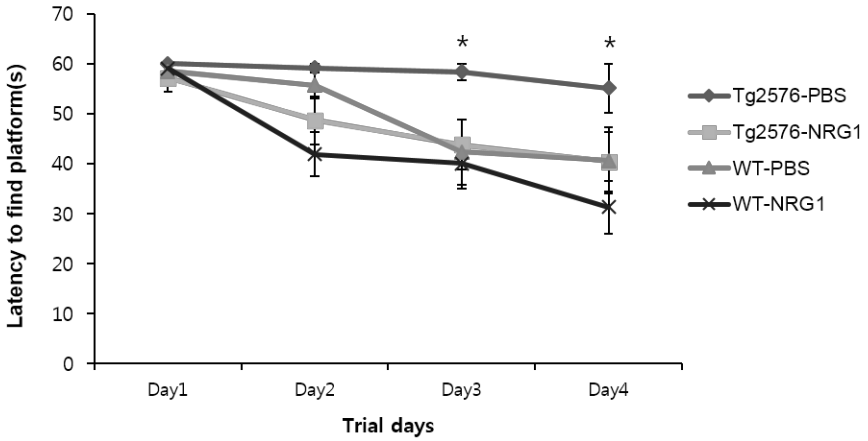
Oligomeric $A\beta_{1-42}$ has been reported to reduce neural differentiation in fetal neural stem cells [56] . Here, I examined whether NRG1 affects the impairment in neural differentiation induced by the treatment with the 500 nM oligomeric $A\beta_{1-42}$ in fetal neural stem cells. Neural stem cells of passage 3 were differentiated in differentiation medium for 3 days. I counted the number of neurons stained with anti-MAP2 or anti-GFAP that were counter-stained with the nuclear marker DAPI. MAP2 stains processes as well as cell bodies. To avoid double counting, MAP2 -positive cells were counted when a MAP2 stained cell body included a DAPI- stained nucleus. In addition, to confirm the effects of ErbB4 at the neural differentiation, the fetal neuronal stem cells were treated with AG1478 and immunostained with anti-GFAP and MAP2 antibodies and immunoreactive cells against GFAP and MAP2 were counted, respectively.

I found that treatment with 500 nM oligomeric $A\beta_{1-42}$ for 3 days significantly reduced neural differentiation (34.45 ± 1.27 % of DAPI positive cells, n= 6, $p < 0.01$), compared to the vehicle-treated control (48.50 ± 3.04 % of DAPI positive cells, n= 6), but increased the number of GFAP-positive cells (43.63 ± 1.69 % of DAPI positive cells,, n= 6, $p < 0.01$), compared to the control (34.87 ± 2.37 %, n= 6). However, co-treatment with 10 nM NRG1 significantly attenuated the reduction in neural differentiation induced by $A\beta_{1-42}$ treatment (45.87 ± 2.52 % of DAPI positive cells, n= 6, $p < 0.01$, Figs. 6A and 6B). In contrast, co-treatment with 10 nM NRG1 significantly reduced the number of GFAP-positive cells (25.48 ± 2.32 % of DAPI positive cells, $p < 0.001$), compared to the 500 nM $A\beta_{1-42}$ plus vehicle treated cells (43.63 ± 1.68 % of DAPI positive cells, n= 6). Treatment with 250 nM AG1478 for 3 days significantly reduced neural differentiation (33.90 ± 1.54 % of DAPI positive cells, n= 6, $p < 0.001$), compared to the vehicle-treated control (48.50 ± 3.04 % of DAPI positive cells, n= 6), but increased the number of GFAP-positive cells (47.79 ± 1.69 % of DAPI positive cells,, n= 6, $p < 0.001$), compared to the control (34.87 ± 2.37 %, n= 6). However, co-treatment with 10 nM NRG1 rescued the reduction in neural differentiation induced by AG1478 (55.10 ± 3.56 % of DAPI positive cells, n= 6, Figs. 6A and 6B). This action of NRG1 is independent with ErbB4.

(A)


~ 12 month
1 week1 day
4 week
Infusion
PBS or NRG1
in subventricular
zone by using
osmotic pump
Probe
test
Water maze test
Molecular study

(B)


Latency to find platform(s)
70
60
50
40
30
20
10
0
Day1
Day2
Day3
Day4
Trial days
*
*
Tg2576-PBS
Tg2576-NRG1
WT-PBS
WT-NRG1

(C)

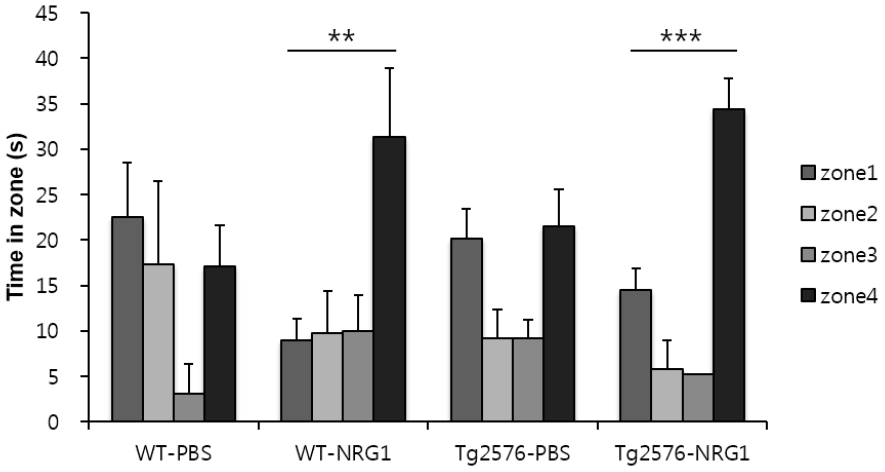
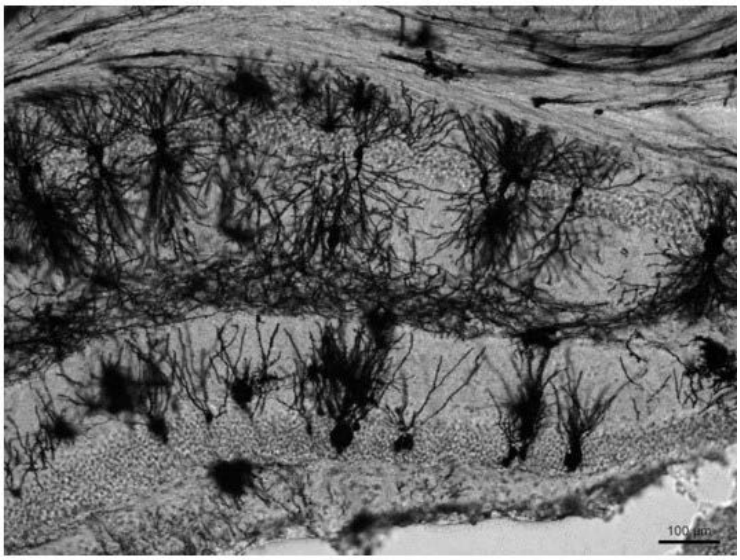

45
40
35
30
25
20
15
10
5
0
Time in zone (s)
**
***
WT-PBS
WT-NRG1
Tg2576-PBS
Tg2576-NRG1
zone1
zone2
zone3
zone4

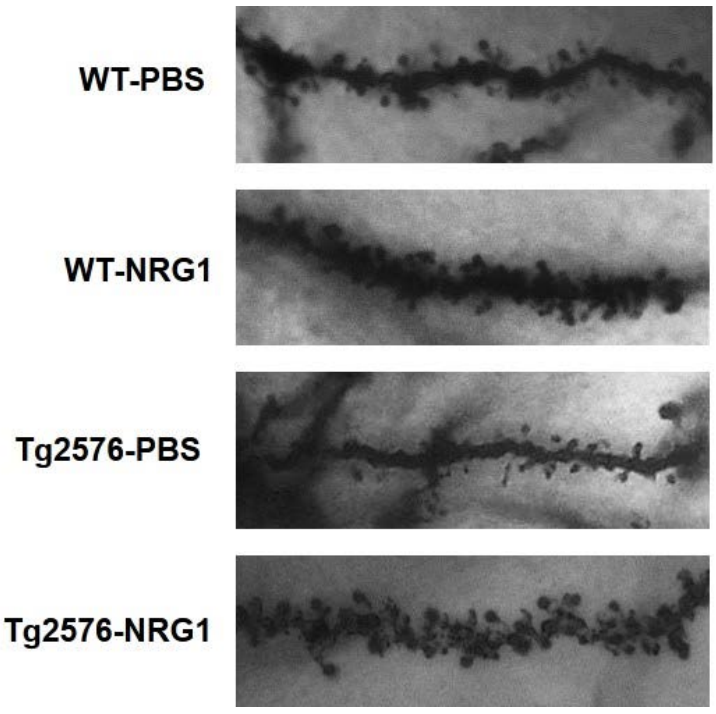
Fig. 1. NRG1 attenuates the impairments in learning and memory in 13-month-old Tg2576 mice

(A) An experimental schematic of intraventricular infusion of NRG1 peptide using an osmotic pump is shown. Vehicle or NRG1 was infused into 12-month-old Tg2576 mice and their WT. The Morris water maze test was performed 4 weeks after the osmotic pump insertion surgery. (B) Animals were required to find a submerged platform (12 cm in diameter, 35 cm in height) in the pool using spatial cues. 3 training trials per day were conducted for 4 consecutive days, in which the initial placement of the mice into the maze was rotated per trial for each group. For each training session, the latency to escape to the hidden platform was recorded. A significant difference was detected between the Tg2576-PBS group and the Tg2576-NRG1 group on day 3 and day 4 of the Morris water maze task. (n= 3 ~ 6) per group; $*p < 0.05$ based on a one-way ANOVA, post-hoc analysis Fisher's LSD. (C) 48 h after the final trial session, a single probe trial was conducted. The escape platform was removed, and each mouse was allowed to swim for 60 s in the maze. NRG1-infused WT or Tg2576 mice remained significantly longer in zone 4 than the other zones (zones 1, 2 and 3) (a one-way ANOVA, $**p < 0.01$, $**p < 0.001$).

(A)



(B)



(C)

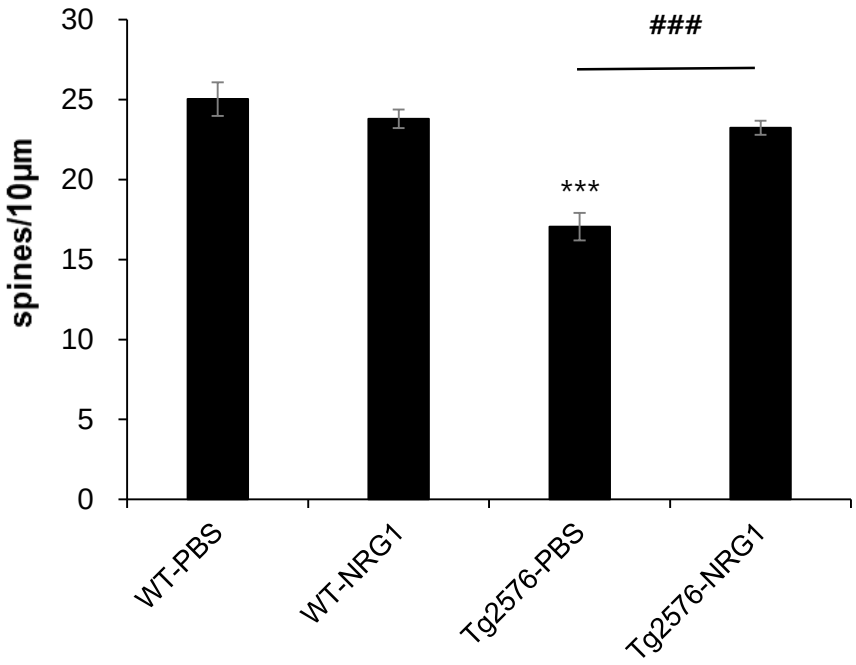

30
25
20
15
10
5
0
spines/10μm
###
***
WT-PBS
WT-NRG1
Tg2576-PBS
Tg2576-NRG1

Fig. 2. NRG1 rescues the reduction in dendritic spine density in Tg2576 mice based on Golgi-Cox staining

(A) Golgi-Cox staining was performed on the mouse brains using a FD Rapid GolgiStain Kit (FD Neuro Technologies, MD, USA) according to the manufacturer's instructions. A representative image of CA1 subregion of hippocampus from WT-PBS-infused mice. Scale bar, 100 μm. (B) Representative images of dendritic spines are provided for the WT-PBS, WT-NRG1, Tg2576-PBS and the Tg2576-NRG1-infused mice. (C) Quantification of the spine density (40 - 80 μm of secondary dendritic spines from the soma) in Tg2576 mice. Tg2576-PBS-infused mice significantly reduced the number of dendritic spines per 10 μm of dendrites (17.06 ± 0.86, n= 11, ***$p < 0.001$), compared to WT-PBS-infused mice (25.03 ± 1.04, n= 23). Tg2576-NRG1-infused mice alleviated the reduction in the number of dendritic spines (23.24 ± 0.44, n= 16, ###$p < 0.001$) compared to Tg2576-PBS-infused mice (17.06 ± 0.86, n= 11). The statistical analysis was performed via a one-way ANOVA with Fisher's LSD *post-hoc* test; the data are represented as the means ± SEM.

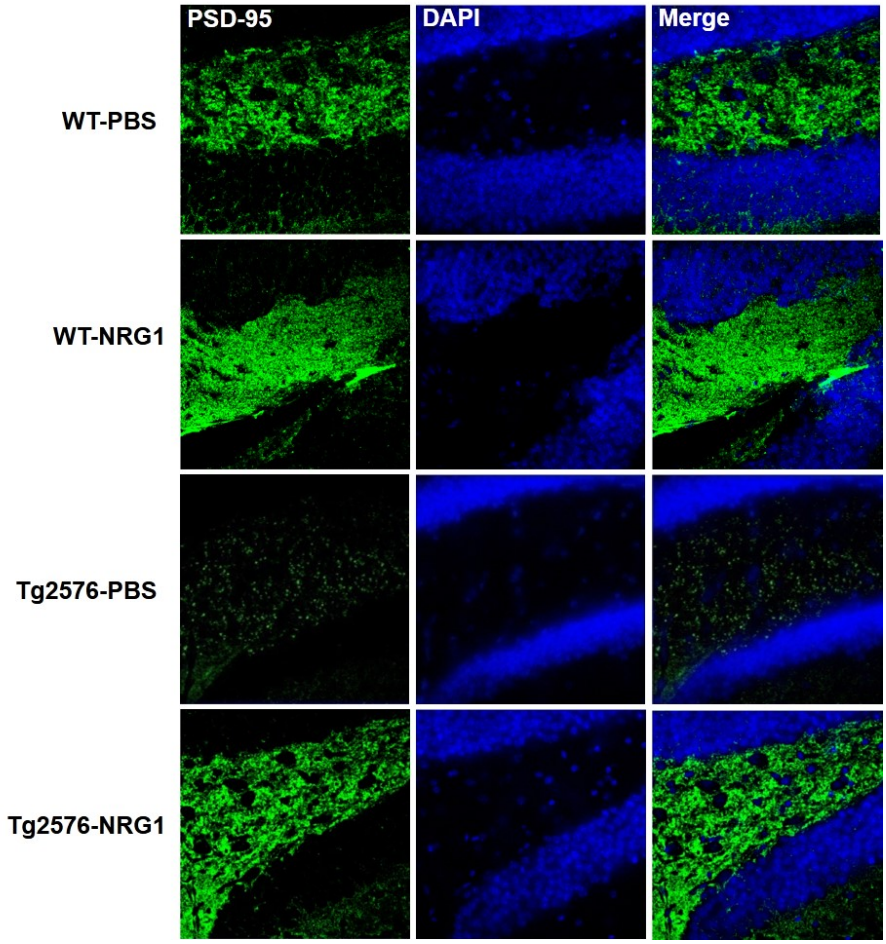
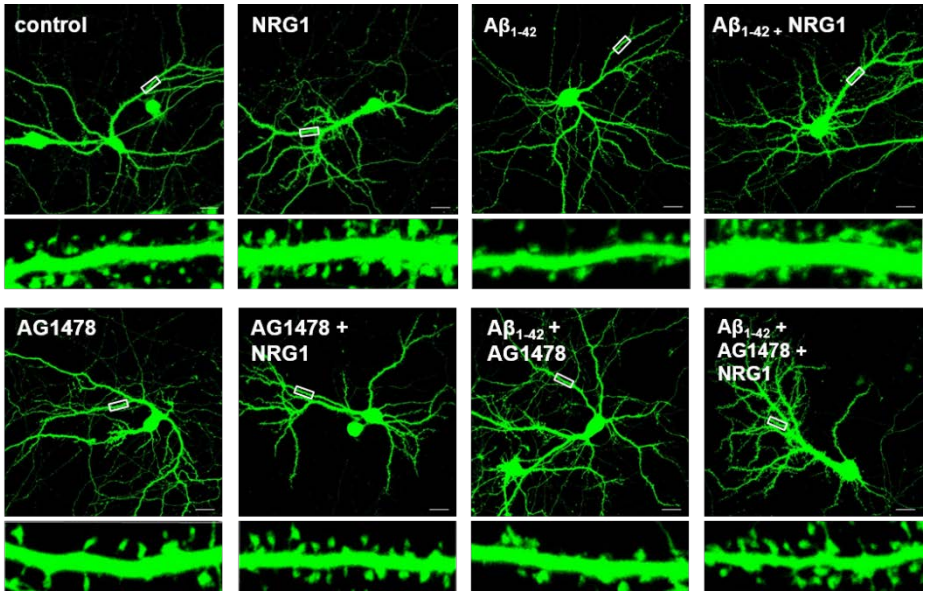

PSD-95
DAPI
Merge
WT-PBS
WT-NRG1
Tg2576-PBS
Tg2576-NRG1

Fig. 3. NRG1 alleviates the reduction in PSD95 immunoreactivity in Tg2576 mice

A coronal section of mouse brain tissues were subjected to immunoperoxidase staining with an anti-PSD-95 antibody. Showing the punctate immunoreactivity of PSD-95 (green) in the dentate gyrus (DG) of subregion of hippocampus for the WT-PBS, WT-NRG1, Tg2576-PBS and the Tg2576-NRG1-infused mice. Nuclei were counterstained with DAPI (blue).

(A)


control
NRG1
Aβ1-42
Aβ1-42 + NRG1
AG1478
AG1478 + NRG1
Aβ1-42 + AG1478
Aβ1-42 + AG1478 + NRG1

(B)

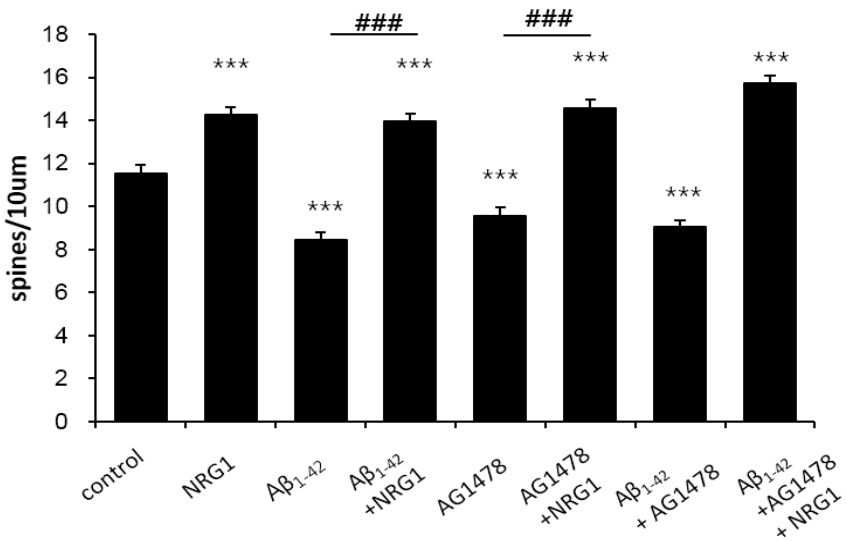
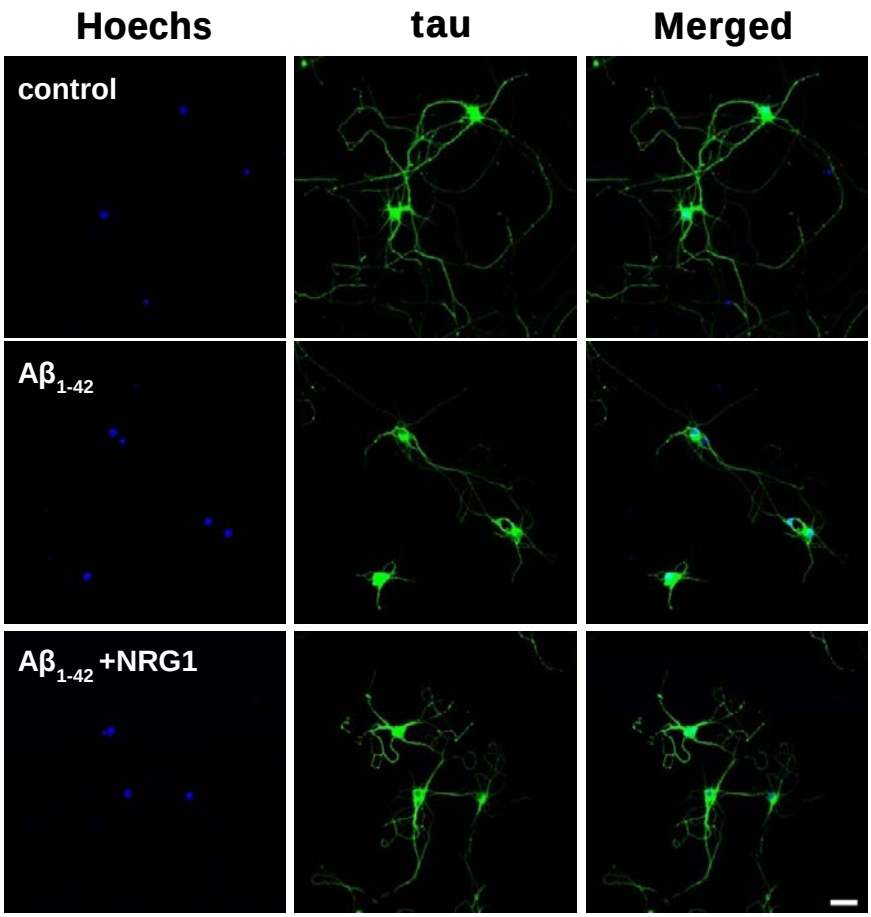

spines/10um
18
16
14
12
10
8
6
4
2
0
###
###
***
control
NRG1
Aβ1-42
Aβ1-42 +NRG1
AG1478
AG1478 +NRG1
Aβ1-42 + AG1478
Aβ1-42 +AG1478 + NRG1

Fig. 4. NRG1 rescues the reduction in dendritic spine density induced by oligomeric $A\beta_{1-42}$ in rat primary hippocampal neuron cultures

(A) Representative images of dendritic spines in primary hippocampal neuron cultures at DIV 17 after treatment with 250 nM oligomeric $A\beta_{1-42}$, (at DIV 13) either alone or with vehicle or NRG1 or AG1478 (at DIV14) were shown. The dendritic segment, outlined with a white box (upper), is magnified to delineate the spine morphology (bottom). The scale bars represent 20 μm (low-scaled panel) and 5 μm (magnified panel). (B) Quantification of the spine density (40 - 80 μm of secondary dendritic spines from the soma) at DIV 17 after transfection of mGFP into cultured primary hippocampal neurons at DIV 12. 250 nM $A\beta_{1-42}$ treatment significantly reduced the number of dendritic spines per 10 μm of dendrites (8.45 ± 0.35, n=16, ***p<0.001), compared to vehicle-treated controls (11.55± 0.388, n=18). Co-treatment with $A\beta_{1-42}$ plus 10 nM NRG1 alleviated the reduction in the number of dendritic spines induced by $A\beta_{1-42}$ (13.96 ± 0.37, n=19, $^{###}p$<0.001) compared to the $A\beta_{1-42}$ treatment (8.45 ± 0.35, n=16). The statistical analysis was performed via a one-way ANOVA with Fisher's LSD *post-hoc* test; the data are represented as the means ± SEM.

(A)


Hoechs
tau
Merged
control
Aβ1-42
Aβ1-42+NRG1

(B)

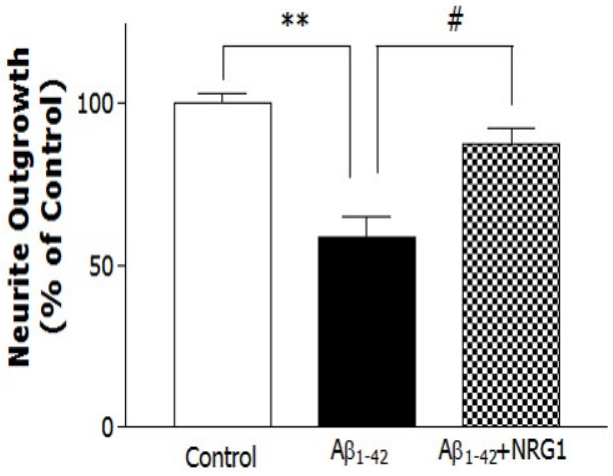
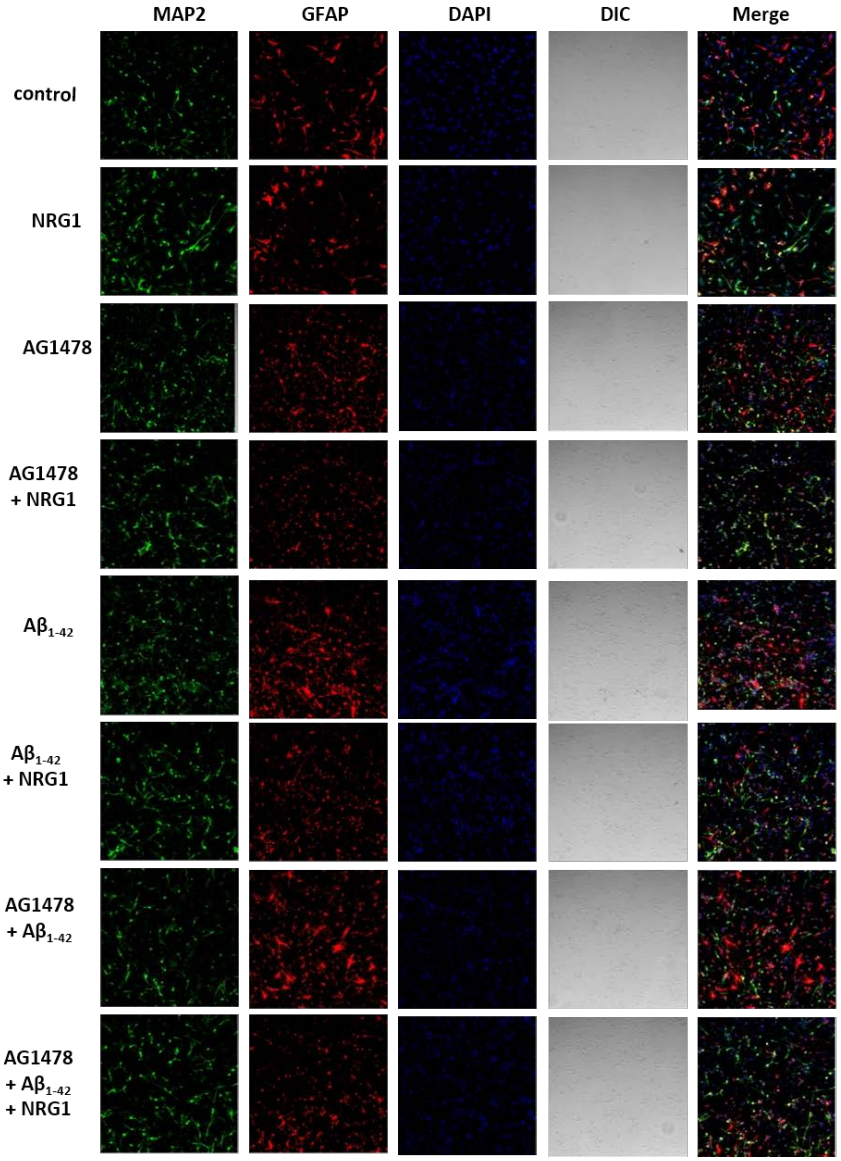

**
#
Neurite Outgrowth (% of Control)
100
50
0
Control
Aβ1-42
Aβ1-42+NRG1

Fig. 5. NRG1 has attenuating effect against oligomeric Aβ1-42 -induced reduction of neurite outgrowth

(A) Representative images of neurons stained with anti-tau antibody followed by visualized with visualized with FITC-coupled secondary antibody were shown. Primary hippocampal neuron cultures were treated with 250 nM oligomeric $A\beta_{1-42}$, (at DIV 13) either alone or with vehicle or NRG1 (at DIV 14). The neurons were fixed and stained with anti-tau antibody, and visualized with FITC-coupled secondary antibody. Scale bar, 100 μm (B) The values of neurite outgrowth are shown as the percentage of the control group (n = 6, $^{**}p < 0.01$, $^{\#}p < 0.05$). Neurite outgrowth in the neurons exposed to oligomeric $A\beta_{1-42}$ (250 nM) for 4 days was reduced significantly (253.73 ± 23.72 μm, n= 6, $^{**}p < 0.01$), compared to the vehicle-treated controls (363.41 ± 10.00 μm, n= 6) by 41.3 %. The co-treatment with 10 nM NRG1 for 3 days restored neurons from oligomeric $A\beta_{1-42}$-induced reduction of neurite outgrowth to 350.72 ± 20.00 μm ($^{\#\#}p < 0.01$, n = 6).

(A)


MAP2
GFAP
DAPI
DIC
Merge
control
NRG1
AG1478
AG1478 + NRG1
Aβ1-42
Aβ1-42 + NRG1
AG1478 + Aβ1-42
AG1478 + Aβ1-42 + NRG1

(B)

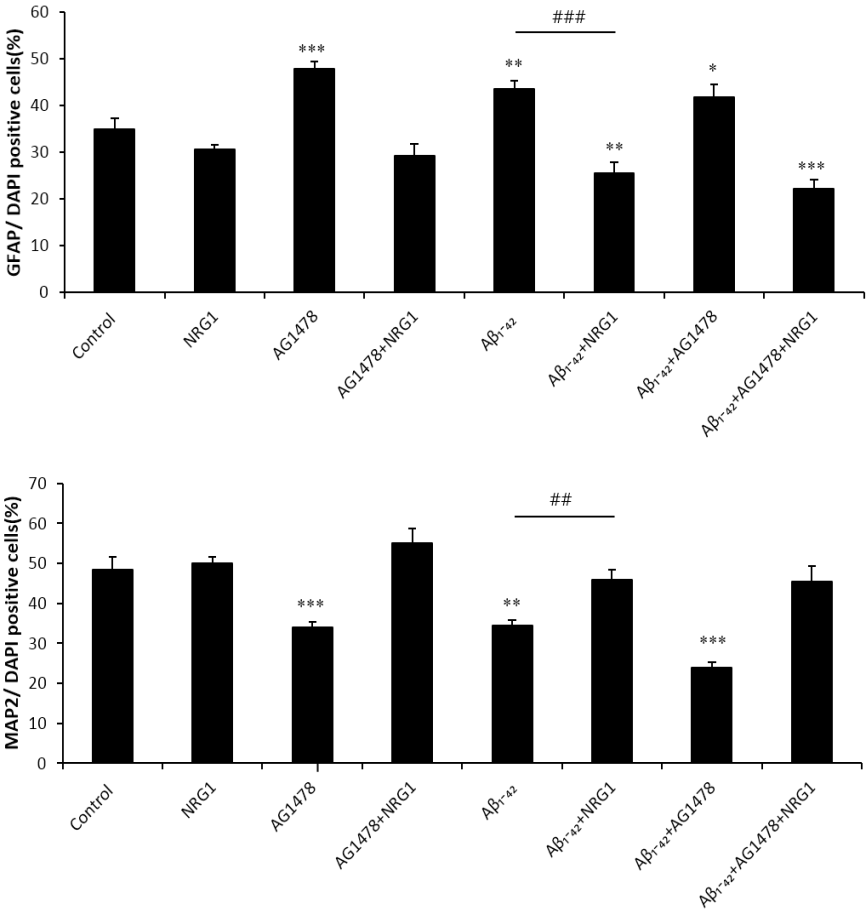

GFAP/ DAPI positive cells(%)
60
50
40
30
20
10
0
###
***
**
**
*
***
Control
NRG1
AG1478
AG1478+NRG1
Aβ1-42
Aβ1-42+NRG1
Aβ1-42+AG1478
Aβ1-42+AG1478+NRG1
MAP2/ DAPI positive cells(%)
70
60
50
40
30
20
10
0
##
***
**
***
Control
NRG1
AG1478
AG1478+NRG1
Aβ1-42
Aβ1-42+NRG1
Aβ1-42+AG1478
Aβ1-42+AG1478+NRG1

Fig. 6. NRG1 attenuates the reduction in neural differentiation induced by oligomeric $A\beta_{1-42}$ in primary fetal neuronal stem cells

(A) Representative immunofluorescence image of cultured fetal neuronal stem cells 5 days after treatment with vehicle or oligomerlic $A\beta_{1-42}$ (500 nM) or AG1478 (250 nM), either vehicle or 10 nM NRG1 at day 2 for 3 days. Cells were fixed on day 5, immunostained for the neuronal marker MAP2 (green) and the astrocytic marker GFAP (red) and counterstained with DAPI (blue). Orthogonal analysis was performed on the stained cells via confocal microscopy. (B) Quantitative graph of MAP2- and GFAP- positive cells (expressed as the percentage of DAPI-positive cells) was shown. The treatment with 500 nM oligomeric $A\beta_{1-42}$ for 3 days reduced neural differentiation (34.45 ± 1.27 %, n= 6, $^{**}p < 0.01$), compared to the vehicle-treated control (48.50 ± 3.04 %, n= 6), but increased the GFAP- positive cells (43.63 ± 1.69 %, n= 6, $^{**}p < 0.01$), compared with the control (34.87 ± 2.37%, n= 6). However, the co-treatment with 10 nM NRG1 significantly attenuated the reduction in neural differentiation induced by $A\beta_{1-42}$ treatment (45.87 ± 2.52 %, n= 6, $^{\#\#}p < 0.01$). In contrast, co-treatment with 10 nM NRG1 significantly reduced the quantity of GFAP-positive cells (25.48 ± 2.32, n= 6, $^{\#\#\#}p < 0.001$), compared to the 500 nM $A\beta_{1-42}$ plus vehicle treated cells (43.63 ± 1.69, n= 6). Data represent the means ± SEM from 2 independent experiments. Treatment with 250 nM AG1478 for 3 days significantly reduced neural differentiation (33.90 ± 1.54 % of DAPI positive cells, n= 6, $p < 0.001$), compared to the vehicle-treated control (48.50 ± 3.04 % of DAPI positive cells, n= 6), but increased the quantity of GFAP-positive cells (47.79 ± 1.69 % of DAPI positive cells,, n= 6, $p < 0.001$),

compared to the control (34.87 ± 2.37 %, n= 6). However, co-treatment with 10 nM NRG1 rescued the reduction in neural differentiation induced by AG1478 (55.10 ± 3.56 % of DAPI positive cells, n= 6). .

DISCUSSION

AD is critically related with age as a risk factor. The amount of neurotrophic factors is known to be reduced with increasing age. In this study, I have assessed the effects of NRG1 *in vitro* and *in vivo* AD experimental models. The infusion of NRG1 using osmotic pump lateral ventricle for 4 weeks rescued the impairments in spatial memory as assessed with Morris water maze test in 13-month-old Tg2576 mice (Fig. 1B). In a probe test, followed by Morris water maze test, WT mouse did not stay at target zone longer than the other zones. Actually, WT mouse found the target zone (zone 4) shortly in probe test however, immediately after the mice realize that the platform did not exist at the location in which the platform existed during training period. Therefore, the mice moved from target zone to another zone to find out the platform. That seemed a problem in a probe test to be modified. In addition, NRG1 rescued the reduction in dendritic spine numbers observed in AD *in vitro* and *in vivo* AD models. In the brain tissues of NRG1 infused Tg2576 mice, the protein levels of PSD 95 immunoreactivity was found to be upregulated in the hippocampus, compared to those of the vehicle-infused mice. In addition, NRG1 has rescued the reduction in neurite outgrowth induced by oligomeric $A\beta_{1-42}$ treatment (Fig. 5). Moreover, NRG1 attenuated the reduction in neural differentiation induced by oligomeric $A\beta_{1-42}$ in primary fetal neuronal stem cells. Collectively, these results suggest that NRG1 possesses a therapeutic potential for AD by upregulating synaptogenesis and neural differentiation of neural stem cells in brains.

In this study, I found out that some discrepancies between the results of the effects of NRG1 on dendritic spine density *in vitro* and *in vivo*. In the brains from WT model with NRG1-infusion, dendritic spine density was not found to be increased (Fig. 2). However, primary hippocampal neuron cultures results showed that NRG1 treatment increased dendritic spine density compared with vehicle-treated neurons (Fig. 4). It has been previously reported that NRG1 inhibits spontaneous firing rates in prefrontal cortex neurons, and also decreases the number of action potentials resulting from a 300 ms current injection [57]. Based on these reports that NRG1 has variable effects *in vivo*, I think that NRG1 might exert different effects from *in vitro*. Therefore, dendritic spine density might not be affected *in vivo* WT model with NRG1-infusion, different from the effects of NRG1 in *in vitro.*

Classically, NRG1 was known to exert its effects by binding to ErbB family members, among them, specifically ErbB4. However, in my results, in inconsistent with my expectations, the treatment with AG1478, an inhibitor for ErbB4 did not prevent the rescuing effects of NRG1 on the reduction in the dendritic spine density or neural differentiation of fetal neural stem cells-induced by oligomeric $A\beta_{1-42}$. According to this results, NRG1 might exert its effects independently of ErbB4 in dendritic spine density and neural differentiation of fetal neural stem cells. The kalirin-7 guanine exchange factor (GEF) plays a key role in regulating structural and functional plasticity at excitatory synapses [58]. Kalirin has been functionally and genetically implicated in the pathogenesis of AD, [59, 60]. Kalirin-7 interacts with ErbB4, and is a critical regulator of NRG1-mediated interneuronal dendritic growth

[61]. NRG1 signaling promotes dendritic spine growth through kalirin [62]. Regarding the detailed mechanisms of effects of NRG1 on the dendritic spine density or neural differentiation in fetal neural stem cell differentiation needs to be assessed.

Historically, the role for acute NRG1 and ErbB4 activity in regulating neuronal function has received much attention, and studies have shown that NRG1/ErbB4 impede synaptic plasticity in pyramidal neurons. NRG1 can reverse LTP at CA1 hippocampal synapses when applied 20 min after theta burst stimulation [63]. In addition to reversing LTP, NRG1 suppresses LTP induction at the Schaffer collateral-CA1 synapse [64]. NRG1 has been shown to inhibit spontaneous firing rates in prefrontal cortex neurons, and also decreases the number of action potentials resulting from a 300 ms current injection [57]. Most of the effects of NRG1 on regulating neuronal function are ErbB4 dependent [57, 64, 65].

In this study, I performed the experiments by treating NRG1 for multiple days. There is a difference between acute and long-term NRG1 activity toward synaptic plasticity, particularly the morphogenesis of dendritic spines on pyramidal neurons, the sites of most excitatory synapses in the brain. Notably, multi-day NRG1 treatment increases spine density and size in cultured forebrain neurons [66], and mice lacking NRG1 type III show a reduction in pyramidal neuron spine density [54]. ErbB4 also has an established role in promoting spine morphogenesis as mice lacking ErbB2/B4 show a reduction in spine density in the CA1 hippocampal field and in the prefrontal cortex [66]. Knocking down ErbB4 with a viral RNA interference (RNAi) in the CA1

hippocampal field reduces spine density and area, while the over-expression of ErbB4 in pyramidal neurons increases spine size [20].

The release of growth factors via proteolytic processing has emerged as an important regulator of many signaling pathways [67, 68] . Dual cleavage of NRG1, which is performed at different two sites located between the EGF-like domain and the transdomain by BACE1 and ADAM 17, respectively, releases its EGF-like domain and promotes paracrine signaling [67]. NRG1 signaling is important for nervous system development, contributing to aspects of neuronal migration, synaptogenesis, gliogenesis, and neuronal–glial communication [69-72]. In mature animals, alterations in NRG1/ErbB signaling manifest as aberrant peripheral myelination, and deficits in synaptic plasticity in hippocampal and cortical slices [20, 54, 63, 73, 74]. NRG1 interacts with ErbB4 to regulate glutamate signaling via the N-methyl-D-aspartate receptor [75].

The NRG1 gene was first identified based on its linkage to chromosome 8p in Icelandic families with schizophrenia [28, 76, 77], followed by the association of this gene with bipolar disorder [78]. Although the underlying mechanism of the pathophysiological involvement in the pathogenesis of schizophrenia remains to be determined, it has recently been reported that NRG1 transgenic mice, which mimic the high levels of NRG1 in forebrain regions of schizophrenic patients, exhibit behavioral deficits and hypofunction of glutamatergic and GABAergic pathways. In addition, this report also demonstrated that LIM domain kinase is involved in the glutamatergic impairments in NRG1 transgenic mice [79]. It appears that the pathological and beneficial roles of NRG1 in the CNS depend on a balance between its

appropriate functions and related signaling pathways. NRG1 has been also reported to be critical for multiple forms of plasticity of cortical projections to pyramidal neurons in the basolateral amygdala, suggesting that loss of NRG1 may disrupt cortex-amygdala neural circuits, resulting in altered processing of salient memories due to the interaction between NRG1 and its ErbBs. This pathway has been implicated in the pathological mechanisms of schizophrenia.

Several lines of evidence has demonstrated that NRG1 protects neurons against toxic stimuli under various conditions including ischemia and exposure to organophosphates [20, 80-83]. NRG1 protected neurons from apoptosis following focal cerebral ischemia via inhibition of caspase-3 and TNF-α expression. It has been shown that the PI3K/Akt pathway is a major contributor to neuronal survival after an ischemic insult [82] . Previously, I have reported that NRG1 exerts neuroprotective effects against neurotoxicity induced by Swedish APP expression and $A\beta_{1-42}$ in neuronal cells via the ErbB4 [65]. Furthermore, NRG1 was found to attenuate the $A\beta_{1-42}$-induced impairment of LTP in hippocampal slices via ErbB4 [84].

AD, the most prevalent neurodegenerative disorder [85], is clinically characterized by the progressive loss of memory and learning ability. A recent report demonstrate that 8 single nucleotide single nucleotide polymorphisms (SNPs) in the NRG3 gene at 10q22-q24 were significantly associated with the risk of AD and SNPs in the NRG3 gene were strongly associated with age at onset of AD, indicating that genetic variants in the NRG3 gene play a role in AD [86].

In this study, I measured the effects of NRG1 in *in vitro* and *in vivo*

experimental models of AD and investigated the underlying mechanism of NRG1 function.

Collectively, our results demonstrate that NRG1 ameliorates cognitive dysfunction in AD via rescuing the reduction in dendritic spine density and neural differentiation in neural stem cells and that NRG1 could be developed as a therapeutic for this disease.

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국문 초록

배경 : 알츠하이머병은 대뇌피질과 해마를 포함한 뇌의 광범위한 부위에서 신경반, 신경섬유 덩어리 및 신경세포의 손상이 특징적으로 나타난다. 알츠하이머병의 동물모델에서는 인지기능과 기억 손상, 수상돌기의 감소, 신경세포의 사멸과 시냅스에 연관된 단백질인 PSD-95와 synaptophysin 등이 감소되어 있다고 밝혀졌다. 최근에는 이러한 현상을 정상으로 회복하게 함으로써 알츠하이머병을 치료할 가능성이 있다는 연구가 진행되고 있다.

표피생장인자 연관 단백질인 뉴레글린(Neuregulin: NRG)은 티로신인산화효소수용체인 ErbB계 단백질과 결합한다. 뉴레글린-1은 신경계에서 말초 미엘린의 형성, 신경근과 감각운동 유지 그리고 대뇌피질 신경 가소성에 있어서 필수적인 기능을 한다.

방법 및 결과 : 본 연구에서는 12개월령의 Tg2576, 알츠하이머병 동물모델에 뉴레글린-1을 뇌실에 4주 동안 삼투압펌프를 이용하여 주입한 후 모리스 수중 회로실험을 통해 인지기능 장애가 유의적으로 완화된다는 것을 증명하였다. 그리고 Tg2576 마우스의 뇌에서 뉴레글린-1 을 주입한 조직과 대조군 뇌조직을 비교하였을 때 감소한 수상돌기 수가 유의적으로 회복되는 것을 Golgi-Cox염색을 통해 확인하였다. 또한 해마 신경세포 배양에서도 뉴레글린-1 은 아밀로이드 베타에 의해 줄어

든 수상돌기 수를 회복시킨다는 결과를 얻었다. 또, 뉴레글린-1 은 아밀로이드 베타에 의해 줄어든 태아 신경줄기세포의 신경세포로의 분화도 유의적으로 회복시켰다.

결론 : 위의 결과를 종합해 보면, 뉴레글린-1 은 알츠하이머병 뇌에서 관찰되는 신경세포의 분화와 수상돌기 수의 감소를 완화시킴으로써 알츠하이머병을 치료할 수 있는 가능성을 가짐을 보여주었다.

주요어 : 알츠하이머병, 뉴레글린-1, 인지기능장애, 신경세포 수상돌기 감소, 신경줄기세포의 신경세포 분화, 시냅스가소성

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