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RESEARCH ARTICLE

Denervated hippocampus provides a favorable microenvironment for neuronal differentiation of endogenous neural stem cells

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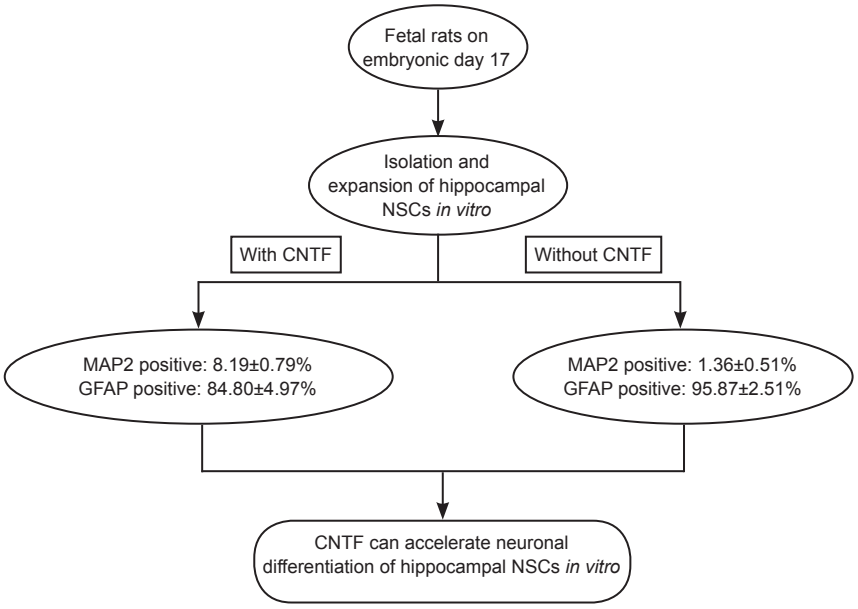
Graphical Abstract

Ciliary neurotrophic factor (CNTF) promotes neuronal differentiation of neural stem cells (NSCs) in the denervated hippocampus



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Abstract

Fimbria-fornix transection induces both exogenous and endogenous neural stem cells to differentiate into neurons in the hippocampus. This indicates that the denervated hippocampus provides an environment for neuronal differentiation of neural stem cells. However, the pathways and mechanisms in this process are still unclear. Seven days after fimbria fornix transection, our reverse transcription polymerase chain reaction, western blot assay, and enzyme linked immunosorbent assay results show a significant increase in ciliary neurotrophic factor mRNA and protein expression in the denervated hippocampus. Moreover, neural stem cells derived from hippocampi of fetal (embryonic day 17) Sprague-Dawley rats were treated with ciliary neurotrophic factor for 7 days, with an increased number of microtubule associated protein-2-positive cells and decreased number of glial fibrillary acidic protein-positive cells detected. Our results show that ciliary neurotrophic factor expression is up-regulated in the denervated hippocampus, which may promote neuronal differentiation of neural stem cells in the denervated hippocampus.

Key Words: *nerve regeneration; ciliary neurotrophic factor; hippocampus; neural stem cells; neurons; neuronal differentiation; fimbria-fornix transection; neural regeneration*

Introduction

Neural circuit damage or neurotransmitter loss induced by neuronal cell degeneration or necrosis in local brain areas is the pathological basis for neurological diseases (Zhang et al., 2014a; Masoudian et al., 2015; McHugh and Buckley, 2015). Currently, the main method for treatment of these diseases is supplementation of neurotransmitters that are reduced because of neuronal loss (Dineley et al., 2015; Levin et al., 2015; Li et al., 2015). However, this approach does not fundamentally solve the problem of neuronal degeneration and necrosis.

Currently, stem cell transplantation for the treatment of nervous system diseases is attracting many researchers' attention (Buzhor et al., 2014; Nicaise et al., 2015; Tong et al., 2015), as stem cells exhibit the potential of multi-lineage differentiation (Komaki et al., 2014; Ren et al., 2015). Neural stem cells (NSCs) are a type of stem cells that are present not only in embryonic tissue (Tsai et al., 2014; Gao et al., 2015) but in adult mammals in the subventricular zone of the lateral ventricles (Boccazzi et al., 2014; Li et al., 2014; Tong et al., 2014), subgranular zone of the dentate gyrus (Clarke and van der Kooy, 2011; Guo et al., 2012; Schultheiß et al., 2013), and even the spinal cord (Xu et al., 2012), striatum (Conway and Schaffer, 2014), and hypothalamus (Rojczyk-Gołębiewska et al., 2014). As these cells exhibit the potential of multi-lineage differentiation, they can differentiate into neurons (Cai et al., 2014; Ramos et al., 2015; Wang et al., 2015), oligodendrocytes (Azim et al., 2014; Shi et al., 2014; Shirazi et al., 2015), and astrocytes (Falcone et al., 2015; Han et al., 2015). Therefore, NSCs are considered to be an ideal cell source to treat neurodegenerative diseases. Research shows that under endogenous conditions, the vast majority of NSCs differentiate into glial cells *in vivo* and *in vitro*, and only a few differentiate into neurons. External factors such as glial cell-derived neurotrophic factor (Deng et al., 2013), retinoic acid (Gu et al., 2015), nerve growth factor, and brain-derived neurotrophic factor (Liu et al., 2014a) more readily promote differentiation of NSCs into neurons, however the number of neurons is too small to meet the requirements of clinical treatment. Thus, investigation of the molecular mechanisms and additional factors involved during NSC differentiation into neurons is urgently needed.

In our previous studies, we established a rat model of hippocampal denervation by fimbria-fornix (FiFx) transection, and transplanted subventricular zone-derived NSCs into the hippocampus of this model. We found that within a certain time period, the implanted NSCs were more likely to survive and differentiate into neurons (Zhang et al., 2007). Next, we cultured hippocampal NSCs obtained from fetal rats with denervated hippocampal extracts, and found that this significantly promoted *in vitro* differentiation of NSCs into neurons (Zhang et al., 2009). These results suggest that during a certain time period after denervation, the hippocampal microenvironment provides favorable conditions for NSCs to survive, regenerate, and differentiate into neurons. Nevertheless, the process remains poorly understood, including the number of factors involved.

Our previous study also found that during a certain time period after denervation, expression of insulin like growth factor-1 and ciliary neurotrophic factor (CNTF) are both up-regulated in the denervated hippocampus. Furthermore, we confirmed that insulin like growth factor-1 induces hippocampal NSCs derived from fetal rats to differentiate *in vitro* into neurons *via* the PI3K/Akt pathway (Zhang et al., 2014b). In this study, cultured rat embryonic hippocampal NSCs were cultured with CNTF *in vitro* to determine if CNTF plays a similar role to insulin like growth factor-1.

Materials and Methods

Ethics statement and animals

Animal studies were approved by the committee for Institutional Animal Care and Use Committee of Nantong University, China, and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Precautions were taken to minimize suffering and the number of animals used in each experiment. Twelve healthy adult female specific pathogen-free Sprague-Dawley rats weighing 200–250 g, and three pregnant Sprague-Dawley rats at embryonic day 17 (E17) were purchased from the Animal Research Center of Nantong University of China (license No. SYXK (Su) 2012-0031).

Establishment of a hippocampal denervation model by FiFx transection

Right FiFx transection was performed as described previously (Zou et al., 2010). In brief, rats were intraperitoneally anesthetized with chloral hydrate (2 mL/kg) and fixed in a stereotaxic instrument (Zhenghua, Anhui Province, China). The skull periosteum was separated, and anterior fontanelle coordinates recorded. According to the atlas of Paxinos and Watson (1986), two points were located on the right side of the skull: anterior (sagittal axis) = 1.4 mm, lateral (coronal axis) = 1.0 mm; and anterior = 1.4 mm, lateral = 4.0 mm (Zou et al., 2010). An aperture was drilled between these two points and a wire-knife lowered to a depth of 5.4 mm ventral to the dura. The knife was shifted back and forth three times before slowly being withdrawn from the brain. The left side of the hippocampus was not operated on, and therefore representative of the normal condition (*i.e.*, control). After surgery, rats were caged with free access to food and water.

Nissl staining

Seven days after surgery, three rats were intraperitoneally anesthetized with chloral hydrate (2 mL/kg) and successively perfused with 0.9% (w/v) NaCl and 4% (w/v) paraformaldehyde. To determine if the right denervated hippocampal model had been successfully established, coronal sections (20 mm) surrounding FiFx were prepared and subjected to Nissl staining. Sections were soaked in dimethylbenzene (two times), 100% alcohol (two times), 95% alcohol, 70% alcohol, and water for 5 minutes each step. Subsequently, sections were stained with 0.1% cresyl violet for 20 minutes, washed with water, and viewed using a phase contrast microscope (Leica, Heidelberg, Germany).

Western blot assay

Whole hippocampi were rapidly removed from three FiFx transected rats. Total protein was extracted from normal and denervated hippocampi using mammalian Protein Extraction Reagent (Pierce, Waltham, MA, USA). Equivalent amounts of protein (30 μg) were loaded and separated on 10% sodium dodecyl sulfate-polyacrylamide gels before transfer to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). Membranes were blocked with 5% non-fat milk in Tris-buffered saline (Sangon, Shanghai, China), incubated overnight at 4°C with primary antibodies (rabbit polyclonal anti-CNTF: 1:1,000, Abcam, Cambridge, UK; mouse monoclonal anti-β-actin: 1:10,000, Sigma, St. Louis, MO, USA), and then secondary antibodies (horseradish peroxidase-conjugated goat anti-mouse IgG, 1:1,000, Pierce; horseradish peroxidase-conjugated goat anti-rabbit IgG, 1:1,000, Pierce) for 2 hours at room temperature. Finally, complexes were visualized by enhanced chemiluminescence (Santa Cruz Biotechnology, Santa Cruz, CA, USA) after X-ray exposure (Kodak, Rochester, NY, USA). The gray scale of each band was scanned and quantified using the Shine-tech Image System (Shanghai, China).

Reverse transcription-polymerase chain reaction (RT-PCR)

Hippocampi were dissected from three FiFx transected rats and total RNA extracted using a Trizol reagent kit (BBI, Markham, Canada). Two μg of total RNA was reverse transcribed into cDNA using oligo (dT) primers and Omniscript reverse transcriptase (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. RT-PCR was performed using the following primers (synthesized by Sangon, Shanghai, China): CNTF: forward, 5′-GGA CCT CTG TAG CCG TTC TA-3′, reverse, 5′-TCA TCT CAC TCC AAC GAT CA-3′; and GAPDH: forward, 5′-ACC ACA GTC CAT GCC ATC AC-3′, reverse, 5′-TCC ACC CTG TTG CTG TA-3′. PCR products were separated by agarose gel electrophoresis and then ethidium bromide stained. Optical density of the CNTF band relative to the GAPDH band was determined using an image analysis system (Leica Q550I W).

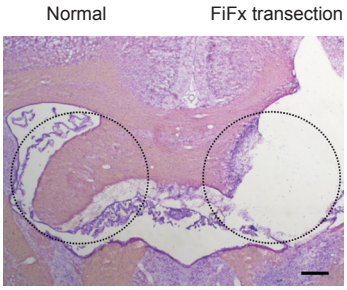

Figure 1 Nissl staining of a coronal brain section showing dissection of the right transected FiFx.
Circles show complete loss of the right FiFx, while the left FiFx remains intact. Scale bar: 300 μm. FiFx: Fimbria-fornix.

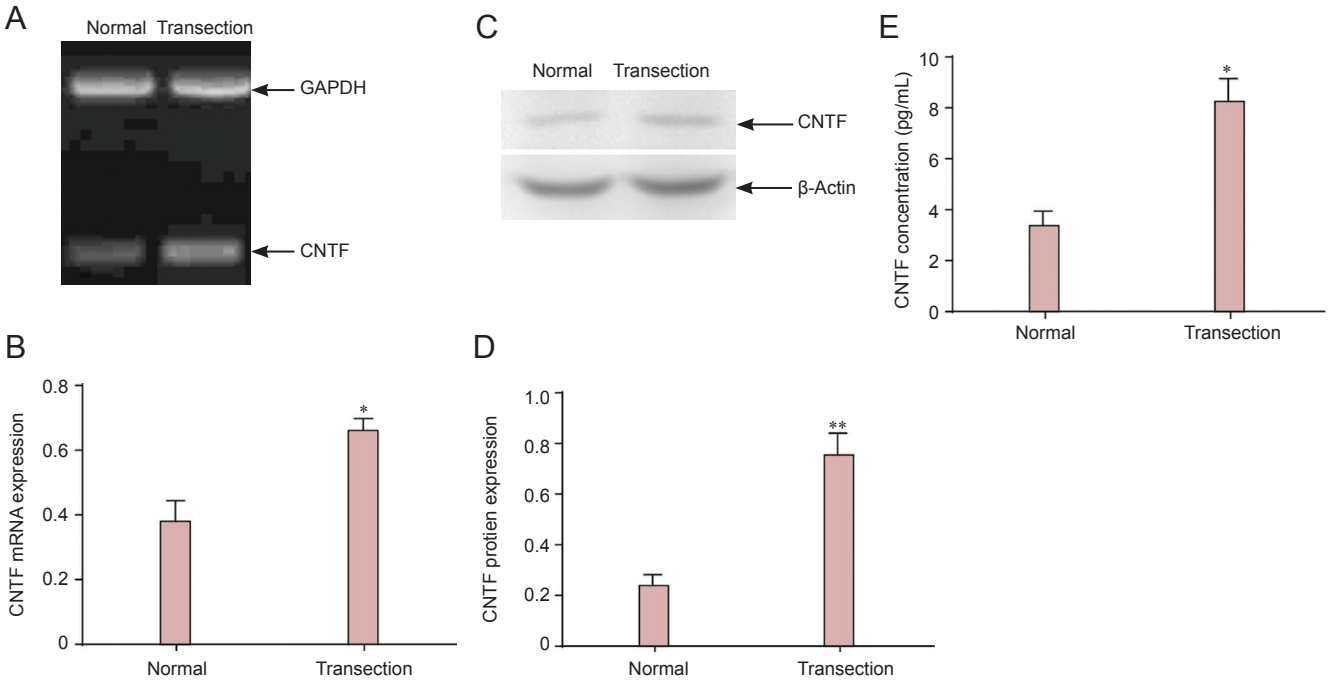

Figure 2 Expression of endogenous CNTF mRNA and protein in normal and the denervated hippocampi 7 days after right FiFx transection.
(A) mRNA levels of CNTF (161 bp) and GAPDH (452 bp, as a reference) detected by RT-PCR. (B) Quantification of CNTF mRNA. Relative expression was expressed as the optical density ratio of CNTF to GAPDH. (C) Western blot assay of protein levels of CNTF and β-actin (as a reference). The molecular weight of CNTF and β-actin are 24 kDa and 42 kDa, respectively. (D) Quantification of CNTF protein. Relative expression was expressed as the optical density ratio of CNTF to β-actin. (E) ELISA identified CNTF protein levels in normal and denervated hippocampi. Data are expressed as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, *vs.* normal hippocampus (two-tailed Student's *t*-test, $n = 3$). CNTF: Ciliary neurotrophic factor; RT-PCR: reverse transcription-polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; ELISA: enzyme linked immunosorbent assay; FiFx: fimbria-fornix.

Enzyme-linked immunosorbent assay (ELISA)
Normal (left) and denervated (right) hippocampi were removed from FiFx transected rats, placed into an aseptic glass-homogenizer with cold Dulbecco's modified Eagle's medium (DMEM, 1 mL/100 mg; Gibco, Grand Island, NY, USA), and homogenized for 10 minutes. Homogenates were centrifuged at 4°C 250 × *g* for 5 minutes. Supernatants were harvested and the amount of CNTF was determined using a rat CNTF ELISA kit (R&D, Minneapolis, MN, USA), according to the manufacturer's instruction.

NSC culture and neuronal differentiation
NSCs were derived from hippocampi of fetal (E17) rats, as described previously (Zhang et al., 2009). In brief, hippocampi were rapidly dissected into 1.5 mL eppendorf tubes containing 0.125% trypsin and mechanically dissociated to single-cell suspensions. These suspensions were centrifuged at 250 × *g* for 5 minutes and the supernatants discarded. Single cells were cultured in 50 cm^2 flasks at a density of 1 × 10^4 cells/mL, with 5 mL NSC culture medium (DMEM/F12 medium (1:1; Gibco), 2% B27 (Gibco), 10 ng/mL epidermal growth factor (Gibco), 10 ng/mL basic fibroblast growth factor (Sigma), and 100 U/mL penicillin/streptomycin) in a humidified 95% air 5% (v/v) CO_2 incubator at 37°C. Five days later, neurospheres were dissociated into single-cell suspensions and seeded into 96-well plates at 1–2 cells per well. Subclonal neurospheres were digested and passaged as before. Cells were passaged three times to obtain neurospheres that originated from single primary cells. On the third passage, neurospheres were rinsed in DMEM and digested into single cells using 0.125% trypsin. Single NSCs were seeded at a density of 5 × 10^5 cells/mL into poly-L-lysine-coated 24-well plates containing differentiation medium (DMEM/F12, 2% fetal bovine serum, and 100 U/mL penicillin/streptomycin). NSCs were cultured without CNTF (control group) or with conditional medium containing 10 ng/mL CNTF (Sigma; CNTF group). After 7 days, differentiation was terminated and cells were detected by immunofluorescence assay.

Immunofluorescence assay
Microtubule associated protein-2 (MAP2) is a marker of mature neurons (Nakano et al., 2015; Razavi et al., 2015) and glial fibrillary acidic protein (GFAP) a marker of glial cells (Babaee et al., 2015; Pacey et al., 2015). Cells were washed twice with ice-cold phosphate buffered saline (PBS), fixed with 100% methanol for 7 minutes at −20°C, and permeated with fresh 4% paraformaldehyde for 20 minutes at room temperature. Cells were blocked with blocking buffer (10% goat serum in PBS containing 0.3% Triton X-100 and 0.03% NaN_3) overnight at 4°C. Next, cells were incubated at 4°C for 24 hours with primary antibody diluted in blocking buffer, followed by incubation overnight at 4°C with secondary antibody diluted in blocking buffer. After washing with PBS, cells were stained with Hoechst 33342 (1:1,000; Pierce) for 30 minutes at room temperature and then viewed using a fluorescence microscope (Leica). The primary antibodies used were: mouse monoclonal anti-MAP2 (1:1,000; Millipore, Boston, MA, USA) and rabbit polyclonal anti-GFAP (1:500; Sigma). The secondary antibodies used were: Alexa Fluor 568-conjugated (red) goat anti-rabbit IgG (1:500; Invitrogen, Carlsbad, CA, USA) and Alexa Fluor 488-conjugated (green) goat anti-mouse IgG (1:200; Invitrogen).

Statistical analysis
Data are expressed as the mean ± SEM. All statistical evaluations were performed using a two-tailed Student's *t*-test, and data were analyzed using SPSS 11.0 software (SPSS, Chicago, IL, USA). A probability level of $P < 0.05$ was considered to be significant.

Results

FiFx transection in the adult rat hippocampus
Nissl staining confirmed complete dissection of the right FiFx, while the left side remained intact (**Figure 1**). This indicates successful establishment of our denervated hippocampal model.

CNTF expression in denervated hippocampus
Seven days after right FiFx transection, CNTF mRNA and protein levels in normal and denervated hippocampi were examined by RT-PCR analysis (**Figure 2A**, **B**), western blot assay (**Figure 2C**, **D**), and ELISA (**Figure 2E**). Compared with normal hippocampus, both CNTF mRNA and protein levels in denervated hippocampus were significantly up-regulated.

Neuronal differentiation of hippocampal NSCs treated *in vitro* with CNTF
After 7 days of differentiation, immunofluorescence showed increased MAP2-positive cell number and decreased GFAP-positive cell number in the CNTF group compared with the control group (**Figure 3**).

Discussion

Recently, an increasing number of studies reported that because of their potential for neuronal differentiation, NSCs are a good cell source for cell therapy of neurodegenerative diseases (Diamandis et al., 2007; Marutle et al., 2007). However, to date, the number of neurons obtained from NSCs using various *in vitro* methods is too small to meet the demands of therapy (Donato et al., 2007; Yi et al., 2008). Thus, to induce differentiation of a sufficient number of neurons from NSCs, and subsequently meet the demands of clinical treatment, further study of the molecular mechanisms underlying neuronal differentiation of NSCs is urgently needed.

Our previous studies have shown that denervating the hippocampus by FiFx transection enables both grafted and endogenous newborn NSCs to proliferate, migrate, and differentiate into neurons in the hippocampus (Zhang et al., 2007; Zou et al., 2010). These indicates that the denervated hippocampus may provide a favorable environment for neuronal differentiation of NSCs. However, the pathways and mechanisms in this process are still unclear.

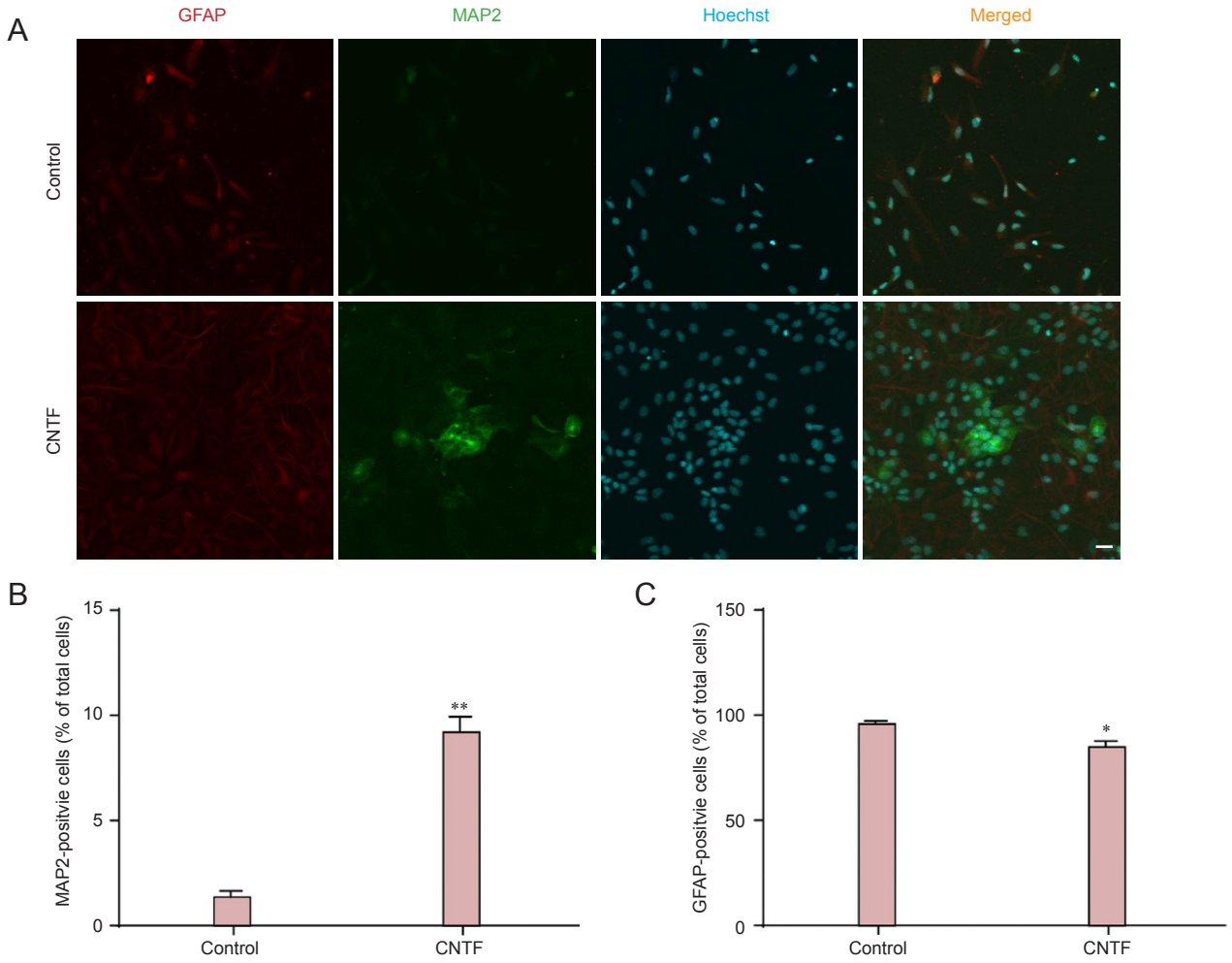

Figure 3 Immunofluorescence analysis of MAP2 and GFAP expression in hippocampal NSCs derived from embryonic day 17 fetal rats.
(A) Cells were separately stained for GFAP (red) and MAP2 (green), with total cell number indicated by Hoechst (blue). Scale bar: 20 μm. (B) MAP2-positive cells are indicated by a percentage of total cells. (C) GFAP-positive cells are indicated by a percentage of total cells. Data are the mean ± SEM. Experiments were performed in triplicate. *$P < 0.05$, **$P < 0.01$, *vs.* control (two-tailed Student's *t*-test). MAP2: Microtubule associated protein-2; GFAP: glial fibrillary acidic protein; CNTF: ciliary neurotrophic factor.

Here, we show that compared with the normal hippocampus, expression of both CNTF mRNA and protein in the denervated hippocampus is significantly up-regulated 7 days after right FiFx transection. CNTF was first reported as a survival factor in ciliary ganglion neurons of chick embryos. It has since been shown that CNTF activates a receptor complex composed of a ligand-binding α-subunit (CNTF Rα) and two signal transducing β-subunits (LIFRβ and gp130) (Inoue et al., 1996). Further studies have shown that CNTF plays a similar role in many other nervous system cells, for example motor neurons (Lamas et al., 2014), sensory neurons (Bailey and Green, 2014), and sympathetic ganglion neurons (Saygili et al., 2011). DeWitt et al. (2014) reported that CNTF promotes neuronal differentiation and cell cycle withdrawal in neuroblastoma cells. Nilbratt et al. (2010) found that CNTF promotes differentiation of human embryonic stem cells into cholinergic neurons. Thus, we hypothesized that increased CNTF in the denervated hippocampus microenvironment might lead to neuronal differentiation of endogenous hippocampal NSCs. To examine this, we cultured hippocampal NSCs derived from E17 fetal rats with exogenous human CNTF for 7 days *in vitro*, and found that approximately 8.19 ± 0.79% cells were MAP2-positive. In contrast, cells cultured in medium without CNTF, resulted in almost no MAP2-positive cells. Instead, the vast majority of cells showed GFAP-positive expression. These results indicate that CNTF promotes a portion of NSCs to differentiate into neurons, while in the absence of CNTF (and any other neurotrophic factors), the vast majority of NSCs spontaneously differentiate into glial cells *in vitro*. Thus, we speculate that CNTF may be one of the factors that promotes neuronal differentiation of endogenous NSCs in the denervated hippocampus. It is still unclear how up-regulated hippocampal CNTF promotes NSC differentiation into neurons after FiFx transection. Many studies have shown two main CNTF-related signaling pathways. Vigneswara et al. (2014) reported that CNTF reduces apoptosis of retinal ganglion cells through the JAK/STAT signaling pathway. Moreover, other studies have shown that CNTF participates in neuronal migration and neurite outgrowth of the dorsal root ganglion through the PI3K/Akt and JAK2/STAT3 signaling pathways

(Liu et al., 2014b). Therefore, in future studies, we will determine if endogenous CNTF promotes neuronal differentiation of hippocampal NSCs in the denervated hippocampus *via* these two signaling pathways *in vivo*. Of course, there may be other signaling pathways involved in this process.

In the present study, the neuronal differentiation efficiency of hippocampal NSCs induced by CNTF *in vitro* was still low. Based on these findings, we speculate that the denervated hippocampus promotes expression of endogenous CNTF and upregulated CNTF may be involved in hippocampal NSC differentiation into neurons *in vivo*. These changes may be beneficial to repair and regeneration of the hippocampus after injury. Although the molecular mechanisms of this phenomenon have not yet been fully confirmed, our findings provide the experimental basis for neuronal differentiation of NSCs with CNTF. These findings may help to promote the clinical application of cell replacement therapy.

Author contributions: *LZ performed western blot assay and wrote the paper. XH completed the immunofluorescence assay and ELISA. XC was responsible for RT-PCR. XFT performed animal experiment. HYZ analyzed the data. XHZ was responsible for study proposal and design. All authors approved the final version of the paper.*
Conflicts of interest: *None declared.*
Plagiarism check: *This paper was screened twice using Cross-Check to verify originality before publication.*
Peer review: *This paper was double-blinded and stringently reviewed by international expert reviewers.*

References

Azim K, Fischer B, Hurtado-Chong A, Draganova K, Cantù C, Zemke M, Sommer L, Butt A, Raineteau O (2014) Persistent Wnt/β-catenin signaling determines dorsalization of the postnatal subventricular zone and neural stem cell specification into oligodendrocytes and glutamatergic neurons. Stem Cells 32:1301-1312.

Babaee A, Eftekhar-Vaghefi SH, Asadi-shekaari M, Shahrokhi N, Soltani SD, Malekpour-Afshar R, Basiri M (2015) Melatonin treatment reduces astrogliosis and apoptosis in rats with traumatic brain injury. Iran J Basic Med Sci 18:867-872.

Bailey EM, Green SH (2014) Postnatal expression of neurotrophic factors accessible to spiral ganglion neurons in the auditory system of adult hearing and deafened rats. J Neurosci 34:13110-13126.

Boccazzi M, Rolando C, Abbracchio MP, Buffo A, Ceruti S (2014) Purines regulate adult brain subventricular zone cell functions: contribution of reactive astrocytes. Glia 62:428-439.

Buzhor E, Leshansky L, Blumenthal J, Barash H, Warshawsky D, Mazor Y, Shtrichman R (2014) Cell-based therapy approaches: the hope for incurable diseases. Regen Med 9:649-672.

Cai M, Zhou Y, Zhou B, Lou S (2014) Hypoxic conditioned medium from rat cerebral cortical cells enhances the proliferation and differentiation of neural stem cells mainly through PI3-K/Akt pathways. PLoS One 9:e111938.

Clarke L, van der Kooy D (2011) The adult mouse dentate gyrus contains populations of committed progenitor cells that are distinct from subependymal zone neural stem cells. Stem Cells 29:1448-1458.

Conway A, Schaffer DV (2014) Biomaterial microenvironments to support the generation of new neurons in the adult brain. Stem Cells 32:1220-1229.

Deng X, Liang Y, Lu H, Yang Z, Liu Re, Wang J, Song X, Long J, Li Y, Lei D, Feng Z (2013) Co-transplantation of GDNF-overexpressing neural stem cells and fetal dopaminergic neurons mitigates motor symptoms in a rat model of Parkinson's disease. PLoS One 8:e80880.

DeWitt J, Pappas A, Nishi R (2014) Ciliary neurotrophic factor reduces proliferation and promotes differentiation of TH-MYCN transformed sympathoadrenal progenitors. Dev Neurosci 36:422-431.

Diamandis P, Wildenhain J, Clarke ID, Sacher AG, Graham J, Bellows DS, Ling EK, Ward RJ, Jamieson LG, Tyers M, Dirks PB (2007) Chemical genetics reveals a complex functional ground state of neural stem cells. Nat Chem Biol 3:268-273.

Dineley KT, Pandya AA, Yakel JL (2015) Nicotinic ACh receptors as therapeutic targets in CNS disorders. Trends Pharmacol Sci 36:96-108.

Donato R, Miljan EA, Hines SJ, Aouabdi S, Pollock K, Patel S, Edwards FA, Sinden JD (2007) Differential development of neuronal physiological responsiveness in two human neural stem cell lines. BMC Neurosci 8:36.

Falcone C, Filippis C, Granzotto M, Mallamaci A (2015) Emx2 expression levels in NSCs modulate astrogenesis rates by regulating EgfR and Fgf9. Glia 63:412-422.

Gao Y, Li X, Zheng D, Guan W, Ma Y (2015) Isolation of a pluripotent neural stem cell from the embryonic bovine brain. Int J Mol Sci 16:5990-5999.

Gu G, Zhang W, Li M, Ni J, Wang P (2015) Transplantation of NSC-derived cholinergic neuron-like cells improves cognitive function in APP/PS1 transgenic mice. Neuroscience 291:81-92.

Guo W, Patzlaff NE, Jobe EM, Zhao X (2012) Isolation of multipotent neural stem/progenitor cells from both the dentate gyrus and subventricular zone of a single adult mouse. Nat Protoc 7:2005-2012.

Han D, Choi M, Jung K, Kim N, Kim S, Chai J, Lee Y, Chai Y (2015) Global transcriptome profiling of genes that are differentially regulated during differentiation of mouse embryonic neural stem cells into astrocytes. J Mol Neurosci 55:109-125.

Inoue M, Nakayama C, Noguchi H (1996) Activating mechanism of CNTF and related cytokines. Mol Neurobiol 12:195-209.

Komaki M, Iwasaki K, Morita I (2014) Bone and Stem Cells. Mesenchymal stem cells and bone regeneration. Clin Calcium 24:565-573.

Lamas NJ, Johnson-Kerner B, Roybon L, Kim YA, Garcia-Diaz A, Wichterle H, Henderson CE (2014) Neurotrophic requirements of human motor neurons defined using amplified and purified stem cell-derived cultures. PLoS One 9:e110324.

Levin ED, Hall BJ, Rezvani AH (2015) Heterogeneity across brain regions and neurotransmitter interactions with nicotinic effects on memory function. Curr Top Behav Neurosci 23:87-101.

Li L, Candelario KM, Thomas K, Wang R, Wright K, Messier A, Cunningham LA (2014) Hypoxia inducible factor-1α (HIF-1α) is required for neural stem cell maintenance and vascular stability in the adult mouse SVZ. J Neurosci 34:16713-16719.

Li Y, Hai S, Zhou Y, Dong BR (2015) Cholinesterase inhibitors for rarer dementias associated with neurological conditions. Cochrane Database Syst Rev 3:CD009444.

Liu F, Xuan A, Chen YAN, Zhang J, Xu L, Yan Q, Long D (2014a) Combined effect of nerve growth factor and brain-derived neurotrophic factor on neuronal differentiation of neural stem cells and the potential molecular mechanisms. Mol Med Report 10:1739-1745.

Liu H, Liu G, Bi Y (2014b) CNTF regulates neurite outgrowth and neuronal migration through JAK2/STAT3 and PI3K/Akt signaling pathways of DRG explants with gp120-induced neurotoxicity in vitro. Neurosci Lett 569:110-115.

Marutle A, Ohmitsu M, Nilbratt M, Greig NH, Nordberg A, Sugaya K (2007) Modulation of human neural stem cell differentiation in Alzheimer (APP23) transgenic mice by phenserine. Proc Natl Acad Sci U S A 104:12506-12511.

Masoudian N, Riazi GH, Afrasiabi A, Modaresi SM, Dadras A, Rafiei S, Yazdankhah M, Lyaghi A, Jarah M, Ahmadian S, Seidkhani H (2015) Variations of glutamate concentration within synaptic cleft in the presence of electromagnetic fields: an artificial neural networks study. Neurochem Res 40:629-642.

McHugh PC, Buckley DA (2015) The structure and function of the dopamine transporter and its role in CNS diseases. Vitam Horm 98: 339-369.

Nakano R, Edamura K, Nakayama T, Narita T, Okabayashi K, Sugiya H (2015) Fibroblast growth factor receptor-2 contributes to the basic fibroblast growth factor-induced neuronal differentiation in canine bone marrow stromal cells via phosphoinositide 3-kinase/Akt signaling pathway. PLoS One 10:e0141581.

Nicaise C, Mitrecic D, Falnikar A, Lepore AC (2015) Transplantation of stem cell-derived astrocytes for the treatment of amyotrophic lateral sclerosis and spinal cord injury. World J Stem Cells 7:380-398.
Nilbratt M, Porras O, Marutle A, Hovatta O, Nordberg A (2010) Neurotrophic factors promote cholinergic differentiation in human embryonic stem cell-derived neurons. J Cell Mol Med 14:1476-1484.
Pacey LK, Guan S, Tharmalingam S, Thomsen C, Hampson DR (2015) Persistent astrocyte activation in the fragile X mouse cerebellum. Brain Behav 5:e00400.
Paxinos G, Watson C (1986) The Rat Brain in Stereotaxic Coordinates. Sydney: Academic Press.
Ramos Alexander D, Andersen Rebecca E, Liu Siyuan J, Nowakowski Tomasz J, Hong Sung J, Gertz CC, Salinas Ryan D, Zarabi H, Kriegstein Arnold R, Lim Daniel A (2015) The long noncoding RNA Pnky regulates neuronal differentiation of embryonic and postnatal neural stem cells. Cell Stem Cell 16:439-447.
Razavi S, Razavi MR, Ahmadi N, Kazemi M (2015) Estrogen treatment enhances neurogenic differentiation of human adipose derived stem cells in vitro. Iran J Basic Med Sci 18:799-804.
Ren Y, Han C, Wang J, Jia Y, Kong L, Eerdun T, Wu L, Jiang D (2015) Identification of genes associated with the differentiation potential of adipose-derived stem cells to osteocytes or myocytes. Mol Cell Biochem 400:135-144.
Rojczyk-Gołębiewska E, Pałasz A, Wiaderkiewicz R (2014) Hypothalamic subependymal niche: a novel site of the adult neurogenesis. Cell Mol Neurobiol 34:631-642.
Saygili E, Pekassa M, Saygili E, Rackauskas G, Hommes D, Noor-Ebad F, Gemein C, Zink MD, Schwinger RH, Weis J, Marx N, Schauerte P, Rana OR (2011) Mechanical stretch of sympathetic neurons induces VEGF expression via a NGF and CNTF signaling pathway. Biochem Biophys Res Commun 410:62-67.
Schultheiß C, Abe P, Hoffmann F, Mueller W, Kreuder AE, Schütz D, Haege S, Redecker C, Keiner S, Kannan S, Claasen JH, Pfrieger FW, Stumm R (2013) CXCR4 prevents dispersion of granule neuron precursors in the adult dentate gyrus. Hippocampus 23:1345-1358.
Shi B, Ding J, Liu Y, Zhuang X, Zhuang X, Chen X, Fu C (2014) ERK1/2 pathway-mediated differentiation of IGF-1-transfected spinal cord-derived neural stem cells into oligodendrocytes. PLoS One 9:e106038.
Shirazi HA, Rasouli J, Ciric B, Rostami A, Zhang G-X (2015) 1,25-Dihydroxyvitamin D3 enhances neural stem cell proliferation and oligodendrocyte differentiation. Exp Mol Pathol 98:240-245.
Tong CK, Chen J, Cebrián-Silla A, Mirzadeh Z, Obernier K, Guinto CD, Tecott LH, García-Verdugo JM, Kriegstein A, Alvarez-Buylla A (2014) Axonal control of the adult neural stem cell niche. Cell Stem Cell 14:500-511.
Tong LM, Fong H, Huang Y (2015) Stem cell therapy for Alzheimer's disease and related disorders: current status and future perspectives. Exp Mol Med 47:e151.
Tsai P-C, Bake S, Balaraman S, Rawlings J, Holgate RR, Dubois D, Miranda RC (2014) MiR-153 targets the nuclear factor-1 family and protects against teratogenic effects of ethanol exposure in fetal neural stem cells. Biology Open 3:741-758.
Vigneswara V, Akpan N, Berry M, Logan A, Troy CM, Ahmed Z (2014) Combined suppression of CASP2 and CASP6 protects retinal ganglion cells from apoptosis and promotes axon regeneration through CNTF-mediated JAK/STAT signalling. Brain 137:1656-1675.
Wang L, Liu Y, Li S, Long ZY, Wu YM (2015) Wnt signaling pathway participates in valproic acid-induced neuronal differentiation of neural stem cells. Int J Clin Exp Pathol 8:578-585.
Xu L, Mahairaki V, Koliatsos VE (2012) Host induction by transplanted neural stem cells in the spinal cord: further evidence for an adult spinal cord neurogenic niche. Regen Med 7:785-797.
Yi SH, Jo AY, Park CH, Koh HC, Park RH, Suh-Kim H, Shin I, Lee YS, Kim J, Lee SH (2008) Mash1 and neurogenin 2 enhance survival and differentiation of neural precursor cells after transplantation to rat brains via distinct modes of action. Mol Ther 16:1873-1882.
Zhang J, Saur T, Duke AN, Grant SG, Platt DM, Rowlett JK, Isacson O, Yao WD (2014a) Motor impairments, striatal degeneration, and altered dopamine-glutamate interplay in mice lacking PSD-95. J Neurogenet 28:98-111.
Zhang X, Jin G, Tian M, Qin J, Huang Z (2007) The denervated hippocampus provides proper microenvironment for the survival and differentiation of neural progenitors. Neurosci Lett 414:115-120.
Zhang X, Jin G, Wang L, Hu W, Tian M, Qin J, Huang H (2009) Brn-4 is upregulated in the deafferented hippocampus and promotes neuronal differentiation of neural progenitors in vitro. Hippocampus 19:176-186.
Zhang X, Zhang L, Cheng X, Guo Y, Sun X, Chen G, Li H, Li P, Lu X, Tian M, Qin J, Zhou H, Jin G (2014b) IGF-1 promotes Brn-4 expression and neuronal differentiation of neural stem cells via the PI3K/Akt pathway. PLoS One 9:e113801.
Zou L, Jin G, Zhang X, Qin J, Zhu H, Tian M, Tan X (2010) Proliferation, migration, and neuronal differentiation of the endogenous neural progenitors in hippocampus after fimbria fornix transection. Int J Neurosci 120:192-200.

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