

RESEARCH ARTICLE



Low-dose curcumin enhances hippocampal neurogenesis and memory retention in young mice

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Abstract

Adult neurogenesis generates new functional neurons from adult neural stem cells in various regions, including the sub-ventricular zone (SVZ) of the lateral ventricles and subgranular zone (SGZ) of hippocampal dentate gyrus (DG). Available evidence shows hippocampal neurogenesis can be negatively or positively regulated by dietary components. In a previous study, we reported that curcumin (diferuloylmethane; a polyphenolic found in curry spice) stimulates the proliferation of embryonic neural stem cells (NSCs) by activating adaptive cellular stress responses. Here, we investigated whether subchronic administration of curcumin (once daily at 0.4, 2, or 10 mg/kg for 14 days) promotes hippocampal neurogenesis and neurocognitive function in young (5-week-old) mice. Oral administration of low-dose curcumin (0.4 mg/kg) increased the proliferation and survival of newly generated cells in hippocampus, but surprisingly, high-dose curcumin (10 mg/kg) did not effectively upregulate the proliferation or survival of newborn cells. Furthermore, hippocampal BDNF levels and phosphorylated CREB activity were elevated in only low-dose curcumin-treated mice. Passive avoidance testing revealed that low-dose curcumin increased cross-over latency times, indicating enhanced memory retention, and an in vitro study showed that low-concentration curcumin increased the proliferative activity of neural progenitor cells (NPCs) by upregulating NF1X levels. Collectively, our findings suggest that low-dose curcumin has neurogenic effects and that it may prevent age and neurodegenerative disease-related cognitive deficits.

Keywords BDNF · CREB · Curcumin · Hippocampal neurogenesis · Memory retention · NF1X · NPCs

Introduction

Neural progenitor cells (NPCs) have the ability to self-renew and proliferate and are capable of differentiating into neurons and glial cells in the brain, and thus, are considered to have therapeutic potential for compensating the neuronal losses in aging and neurodegenerative diseases (Einstein and Ben-Hur 2008). NPCs exist in all brain regions during the embryonic stage, but in the adult brain they are limited to the subgranular zone (SGZ) of the dentate gyrus (DG) and subventricular zone (SVZ) of lateral ventricles (Eriksson et al. 1998). Adult hippocampal neurogenesis was also observed in the adult human hippocampus (Eriksson et al. 1998) and results in the generation of functional neurons from neural stem cells (NSCs) and provides structural and functional plasticity in the SGZ of the hippocampal DG (Toda and Gage 2018). The continued neurogenesis in adult brain, particularly in the hippocampus, which plays an important role in learning

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and memory, is needed to maintain neurocognitive functions and memories (Toda et al. 2019; Lim et al. 2021). In adulthood, there is a constant turnover of interneurons and granule cells, and newly generated neurons replace dying cells and successfully form functional synapses (van Praag et al. 2002). This hippocampal neurogenesis in adult stage is known to be influenced by aging, stress (Babcock et al. 2021; Leschik et al. 2021), an enriched environment, physical exercise, and growth factors (Ma et al. 2017; Zhang and Zhang 2018; Gronska-Peski et al. 2021). Interestingly, dietary restriction (DR) has been known to enhance hippocampal neurogenesis by regulating levels of brain-derived neurotrophic factor (BDNF) (Lee et al. 2000, 2002a, 2002b), and recent studies have shown dietary components and dietary modulation affect hippocampal neurogenesis and neurocognitive functions (Bracko et al. 2020; Ferragud et al. 2020; Frausto et al. 2021; Paulo et al. 2021; Lu et al. 2022; Zhou et al. 2022).

In a previous study, we found that low-concentration of curcumin (at 500 nM) stimulated C17.2 NPCs and mouse cortical NSCs cultures via extracellular signal-regulated kinase (ERK) and p38 MAP kinase-mediated mechanisms (Kim et al. 2008). Curcumin (diferuloylmethane) is a polyphenolic derived from *Curcuma Longa* Linn. and a yellow component of turmeric, which is considered a treatment for injury and inflammation (Hewlings and Kalman 2017; Razavi et al. 2021). A previous review reported that the beneficial effects of curcumin on cancer and inflammation are due to its ability to scavenge free radicals and suppress inflammation (Razavi et al. 2021), and it was recently suggested that its antioxidant activity might be useful for treating the neuroinflammation and cognitive deficits associated with neurodegenerative diseases and aging (Queen and Tollefsbol 2010; Gomez-Pinilla and Nguyen 2012; Mythri and Bharath 2012). Furthermore, curcumin is highly lipophilic and may cross the blood-brain barrier (BBB) and cross the brain (Kelloff et al. 1996). It was also reported that curcumin modulates multiple cellular targets and has therapeutic potential against a broad range of diseases, including inflammatory and cardiovascular diseases, diabetes, obesity, neurodegenerative diseases, and cancer (Jamwal 2018). Therefore, curcumin is currently marketed as a dietary supplement, such as a spice and health supplement in many countries. Although curcumin is edible, our previous study showed it has neurogenic effects when acute administered intraperitoneally (once daily at 500 nmole/kg for 4 days) (Kim et al. 2008). However, the effects of long-term curcumin intake on hippocampal neurogenesis are unknown. In the present study, we examined whether

subchronic oral administration of curcumin (once daily for 14 days) affects hippocampal neurogenesis and explored the mechanism involved.

Materials and methods

Materials

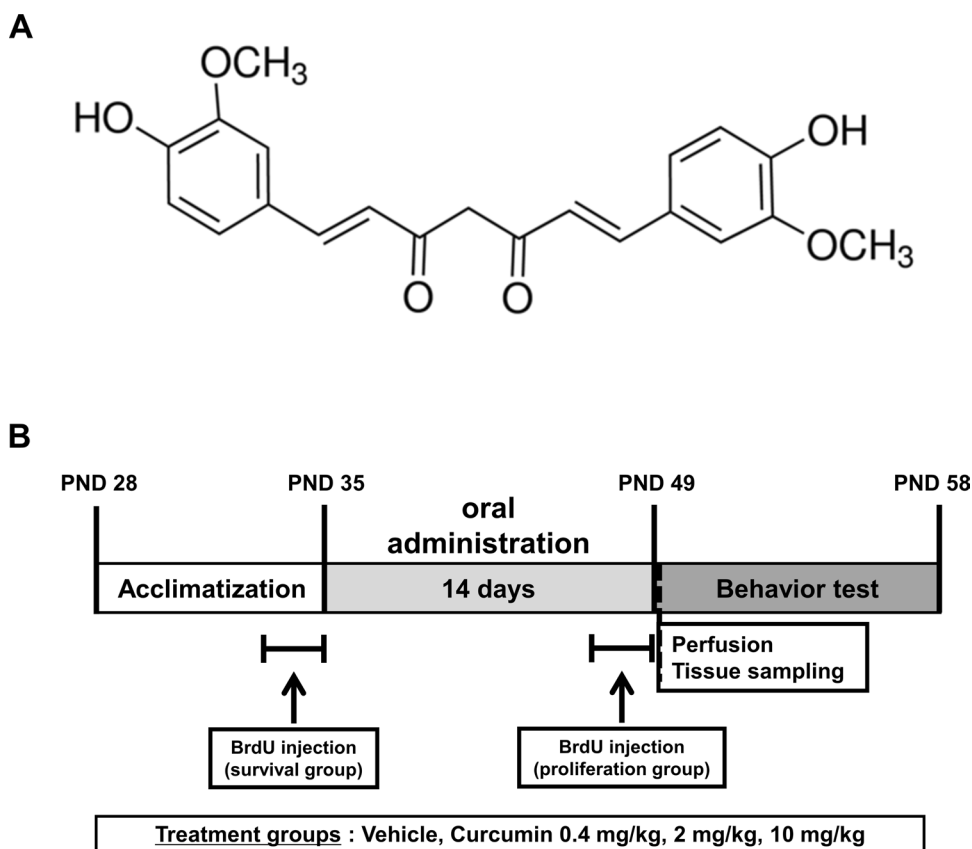
Curcumin, 3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyltetrazolium bromide (MTT) was obtained from the Sigma Chemical Company (St. Louis, MO, USA), 5'-bromo-2'-deoxyuridine (BrdU) was purchased from ACROS organics (Fair Lawn, NJ, USA), and Western blot detection reagent (ECL solution) was obtained from Advansta Inc. (Menlo Park, CA, USA). Alexa Flour 488 and 568 were purchased from Invitrogen (Eugene, OR, USA).

Experimental animals

Four weeks old male C57BL/6 mice were obtained from Daehan Biolink Co. Ltd. (Chungbuk, South Korea) and acclimatized under temperature- and light-controlled conditions (20–23 °C, 12 h/12 hr light/dark cycle). Food and water are provided *ad libitum*. Mice were randomly divided into three groups (proliferation, survival, or behavior groups) containing 5–10 animals per group and acclimatized for one week prior to curcumin administration. Curcumin was dissolved in PBS containing 2% Tween 20 and 5% ethanol and administered orally (p.o.; *per os*) at 0.4, 2, or 10 mg/kg daily for 14 days. Same volume of PBS containing 2% Tween 20 and 5% ethanol administered to mice in the vehicle group. To evaluate new cell proliferation, mice were administered six i.p. injections of BrdU (100 mg/kg twice daily on the last three days of curcumin administration), and in order to evaluate how many newborn cells were survived, mice were administered BrdU for 3 days prior to curcumin or vehicle administration. Mice were euthanatized one day after the final BrdU injection (day 15). The *in vivo* experimental scheme is shown in Fig. 1. The institutional animal care committee of Pusan National University approved the experimental protocol (#PNU-2019-2399).

Fig. 1 The chemical structure of curcumin and a schematic of the in vivo experimental procedure.

A The chemical structure of curcumin. **B** C57BL/6 mice (5-week-old) were administrated vehicle or curcumin orally for 14 days. In the proliferation group, BrdU pulses were given on the last 3 days of vehicle or curcumin administration, whereas in the survival group, BrdU pulses were given 3 days before vehicle or curcumin treatment



Brain tissue preparation

For histological analysis, mice were anesthetized and perfused intracardially with 4% paraformaldehyde (PFA) in 0.1 M PBS (pH 7.4). After fixative perfusion, whole brains were removed, placed in the same fixative at 4 °C overnight, and transferred to a 30% sucrose solution. Cryoprotected brains were sectioned serially at 40 µm in the coronal plane using a freezing microtome (MICROM, Germany) and stored at 4 °C in Dulbecco's phosphate-buffered saline (DPBS) solution containing 0.1% sodium azide. For biochemical analysis, hippocampi were dissected, snap-frozen in liquid nitrogen, and kept at −80 °C until required for analysis. The hippocampal tissues were homogenized in a buffer (50 mM Tris buffer, pH 8.0) containing 6 mM MgCl₂, 1 mM EDTA, 10% sucrose, 1% SDS, and protease inhibitor cocktail (Roche, Mannheim, Germany).

Immunohistochemistry

Staining for newly-generated cells in the DG was performed by treating free-floating brain sections with 0.6% H₂O₂ in TBS (pH 7.5) for 30 min to block endogenous peroxidase

activity, and then DNA was denatured by exposing sections sequentially to heat (65 °C) for 2 h, acid (2 N HCl) at 37 °C for 30 min, and 0.1 M borate buffer at room temperature for 15 min. Sections were in blocking solution (TBS/0.1% Triton X-100/3% goat serum (TBS-TS)) for 30 min, and then treated with primary anti-BrdU antibody (rat; 1:500; Abcam) or anti-pCREB antibody (rabbit; 1:500; Cell Signaling) in TBS-TS overnight at 4 °C. Sections were incubated with biotinylated secondary goat anti-rat or rabbit IgG antibody (Vector Laboratories) for 3 h and then with ABC solution (Vectastain ABC reagent Elite Kit, Vector Laboratories) for 1 h at room temperature. They were stained with diaminobenzidine (DAB) solution for 5 min, mounted, and cover slipped. Images were acquired using a Nikon ECLIPSE TE 2000-U microscope. Cells in every sixth section throughout the entire rostro-caudal region of the hippocampus were counted. The granular cell layer of the DG was used as the reference region. All cell counts were performed by the same investigator unaware of mouse treatment histories.

Double-label immunohistochemistry

Brain sections were incubated in blocking solution for 30 min and treated with primary antibodies against BrdU (the proliferation marker; rat monoclonal; Abcam) and NeuN (the neuronal marker; mouse monoclonal; Millipore Corporation) or DCX (doublecortin, the immature neuronal marker; rabbit polyclonal; Cell Signaling Technology) overnight at 4 °C. Sections were washed with TBS and incubated for 3 h with anti-rat IgG conjugated with Alexa Fluor 488 and anti-mouse (or rabbit) IgG conjugated with Alexa Fluor 568. Images were acquired using a FV10i FLUOVIEW Confocal Microscope (Olympus, Tokyo).

BDNF ELISA assay

Levels of BDNF protein were measured by using a commercially available kit (Millipore Corporation). Briefly, hippocampal homogenates (100 µl) were added to the wells pre-coated with rabbit anti-human BDNF polyclonal antibody. Plates were incubated overnight at 4 °C, washed with washing buffer, and biotinylated mouse anti-BDNF monoclonal antibody (100 µl) was added to each well for 2 h at room temperature. Plates were then washed with washing buffer, and incubated for 1 h in streptavidin-HRP conjugate solution (100 µl). Finally, TMB/E substrate (100 µl) was added to each well and plates were incubated for 15 min. The reaction was stopped by adding 100 µl of stop solution causing the blue reaction solution to turn yellow. Absorbance was measured at 450 nm using a plate reader.

Western blot analysis

After experimental treatments, tissue homogenates were determined using a Bio-Rad (Hercules, CA) protein assay kit. Total proteins (20 µg per lane) were then loaded in 10% SDS-polyacrylamide gels and electrophoretically transferred to Immobilon-P^{SQ} transfer membranes (Millipore, Bedford, MA). Membranes were immediately placed in a blocking solution (5% nonfat milk) at room temperature for 30 min and then incubated with primary antibodies: DCX (rabbit; 1:1,000; Cell Signaling Technology), NF1X (rabbit; 1:500; Abcam) and β-actin (mouse; 1:10,000; Sigma) in TBS-T (Tris-HCl-based buffer with 0.2% Tween 20, pH 7.5) at 4 °C overnight. Membranes were washed for 10 min and incubated with secondary antibody, polyclonal anti-rabbit antibody, or monoclonal anti-mouse antibody (1:10,000; Santa Cruz Biotechnology) in TBS-T buffer at room temperature for 2 h. Horseradish peroxidase (HRP)-conjugated secondary antibody labeling was detected by enhanced chemiluminescence (ECL) and using a cooled

CCD camera system ATTO Ez-Capture (Atto Corp., Tokyo, Japan). Blots were quantified by densitometry.

Morris water maze behavior test

The Morris water maze test was conducted as previously described (Shukitt-Hale et al., 2007). This spatial reference memory test is commonly used to examine spatial learning and memory and utilizes a paradigm that requires mice to find a hidden platform (10 cm in diameter) just below the surface (1 cm) of a circular pool of opaque water (120 cm in diameter 32 cm high, maintained at 23–25 °C). Mice were given six consecutive days of training (six trials/day) after 14 days of vehicle or curcumin treatment. Mice were allowed 60 s to find the platform; mice that failed to find the platform after 60 s were guided to the platform by the investigator. After finding the platform, mice were allowed to remain in position for 10 s. At the end of each trial, mice were towel-dried and returned to their home cages for approximately 15–20 min before being returned to the maze for the next trial. Probe trials were performed to assess spatial reference memory 24 h after last acquisition trials. For probe trials, the platform was removed from the pool and mice were introduced into the water at a new entry point. Mice were allowed to swim freely for 60 s and videotaped. Swim paths were recorded using image tracking software (Noldus EthoVision[®] XT, Noldus Information Technology, Wageningen, Netherlands) and used to determine times taken (latencies) to find the platform (s), path lengths (cm), and swimming speeds (cm/s).

Passive avoidance test

Passive avoidance test was performed using a Gemini Avoidance System (San Diego Instrument, San Diego, CA). Passive avoidance learning memory was investigated using the step-through test, which is based on mice's preference for dark environments. The device consists of illuminated (bright) and dark compartments. For the single training day, mice were placed in the illuminated compartment and received an electric foot shock (0.25 mA, 2 s) as they entered the dark compartment. On test days (1 day or 7 days post training), mice were placed in the illuminated compartment for a maximum of 300 s, and step-through latencies (times taken to enter the dark compartment) were recorded. No shock

Fig. 2 Low-dose of curcumin increased hippocampal neurogenesis in C57BL/6 mice.

A Representative images showing BrdU-positive cells in the DG. Representative hippocampal sections (8–10) were immunostained with BrdU-specific antibodies. Scale bar = 100 μ m. **B** The graph shows numbers of BrdU-positive cells in the DG ($n=5-6$ mice/group). Data are presented as means $\pm$ SEMs. $*p<0.05$ vs. Vehicle. Low-dose curcumin increased the proliferation and survival of newly generated cells in the DG. **C** Double labeling immunohistochemistry was performed with primary antibodies against BrdU (green) and NeuN (mature neuron marker; red) to determine the effect of curcumin on hippocampal neurogenesis. Scale bar = 100 μ m. **D** Graph showing the percentages of BrdU- and NeuN-positive cells in the hippocampal DG ($n=5-6$ mice/group). Data are presented as means $\pm$ SEMs. $*p<0.05$ vs. Vehicle. **E** Double labeling immunohistochemistry was performed with primary antibodies against BrdU (green) and DCX (immature neuron marker; red) to determine the effect of curcumin on neuronal differentiation. Scale bar = 100 μ m. **F** Western blot was performed using homogenates of hippocampal tissues, and findings were confirmed quantitatively. Data are presented as means $\pm$ SEMs. $*p<0.05$ vs. Vehicle

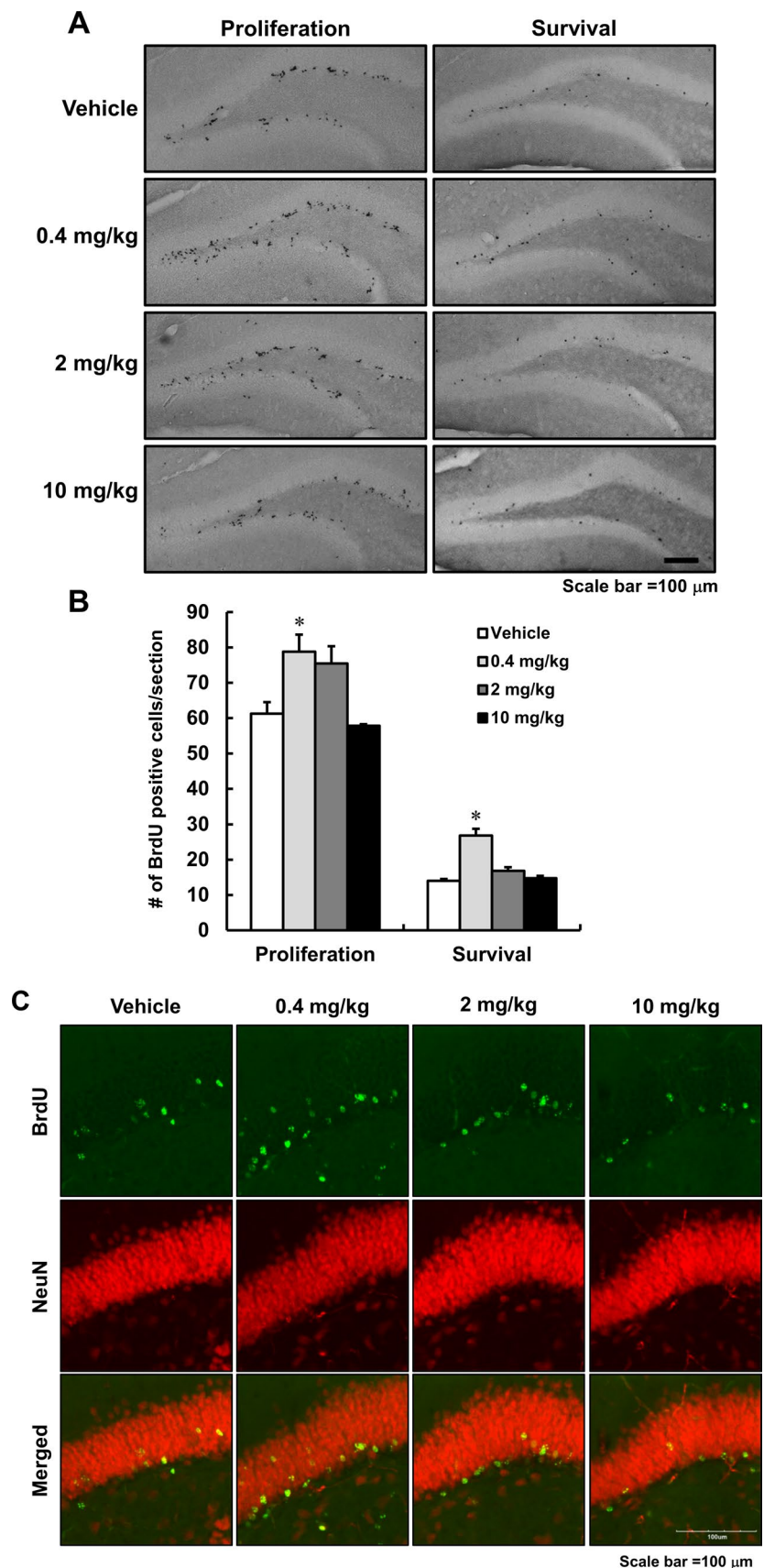


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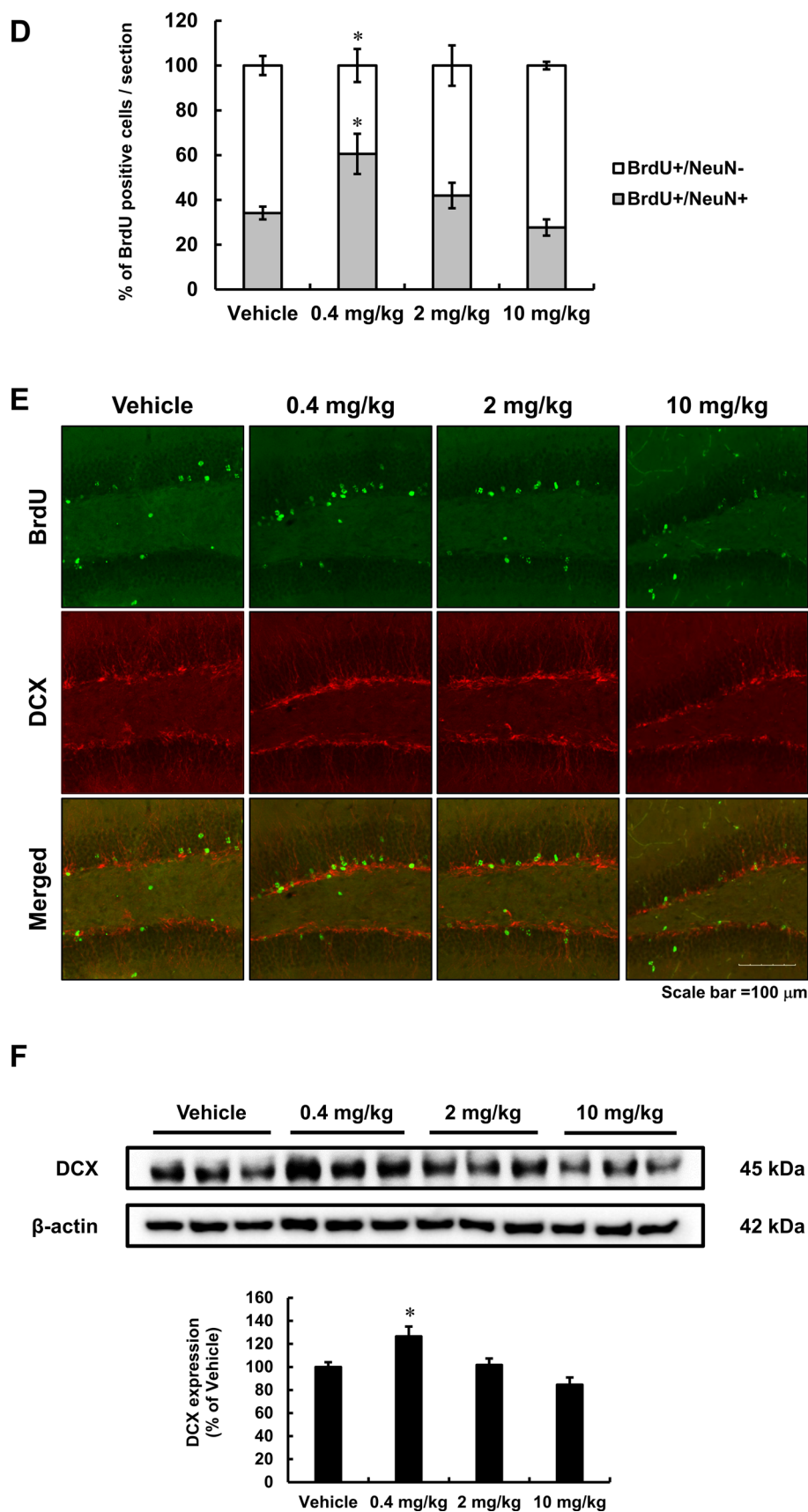
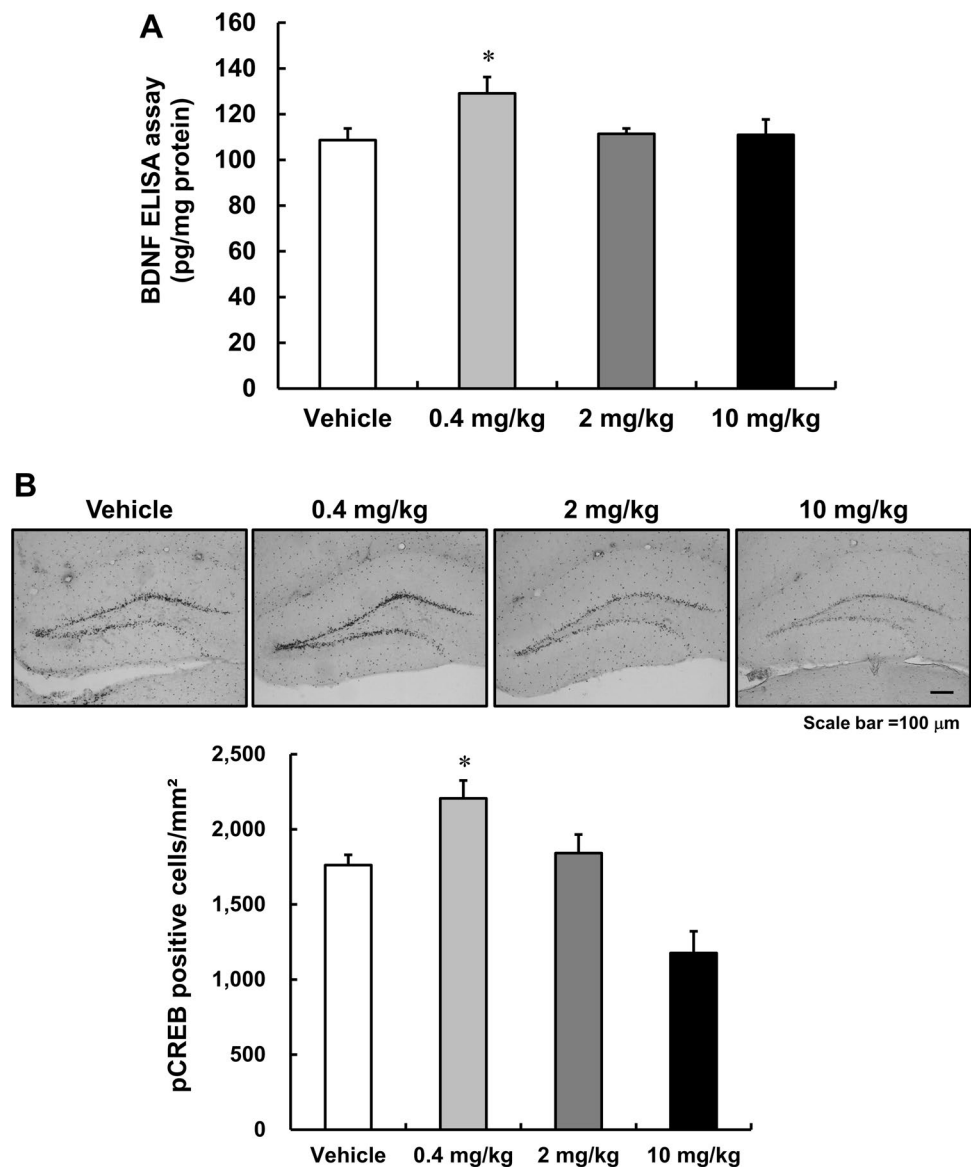


Fig. 3 Low-dose curcumin increased hippocampal BDNF and pCREB levels. **A** BDNF protein levels in hippocampal homogenates from vehicle or curcumin-treated mice were measured by using a quantitative ELISA assay. Data are presented as means $\pm$ SEMs. $*p < 0.05$ vs. Vehicle. Low-dose curcumin (0.4 mg/kg) increased BDNF protein levels in hippocampus. **B** Reductions in pCREB levels in the hippocampal DG of vehicle and curcumin-treated mice were evaluated immunohistochemically. Quantitative analysis of pCREB-positive cell numbers in DG was shown ($n = 5-6$ mice/group). Data are presented as means $\pm$ SEMs. $*p < 0.05$ vs. Vehicle. Scale bar = 100 μ m



was administered during test days. Latency was used as a measure of memory retention.

Culture and experimental treatment of C17.2 NPCs

C17.2 NPCs (a generous gift from C. Cepko at Harvard University) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 5% horse serum, and 2 mM glutamine in a humidified 5% CO₂/95% air atmosphere at 37 °C. Cells (1×10^4 cells/ml) were seeded in 96-well plates for 24 h and then treated with different concentrations

of curcumin (0.5, 2, 10, 20, or 100 μ M). After removing media, cells were washed twice with PBS, 200 μ l of a 0.5 mg/ml MTT solution in PBS was added per well, and plates were incubated for 4 h at 37 °C. The MTT solution was then removed, and the cells were lysed in a solubilization solution (1:1 dimethyl sulfoxide:ethanol). Amounts of formazan released were quantified using an ELISA microplate reader at 560 nm.

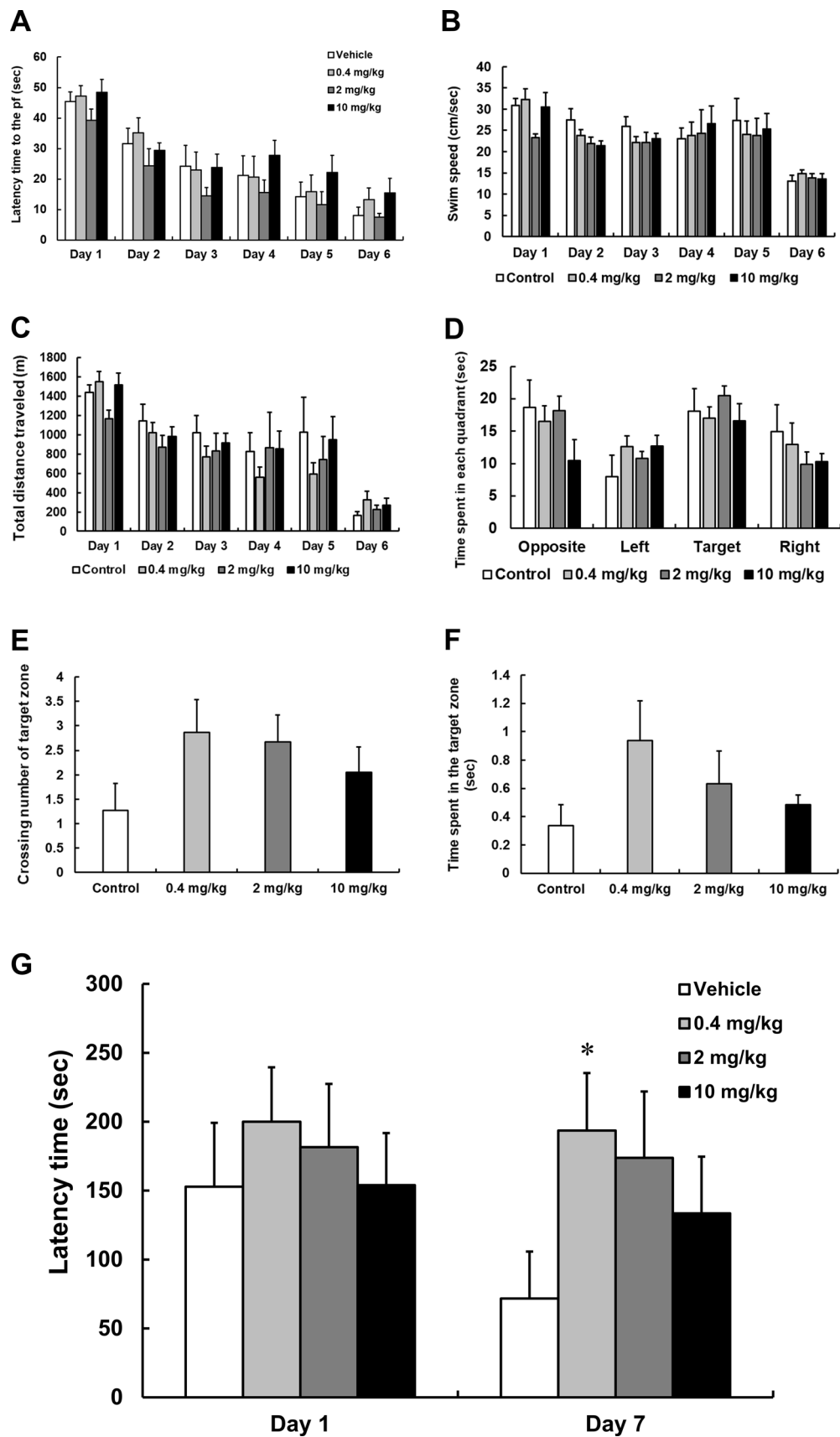


Fig. 4 Effects of curcumin on hippocampus-dependent learning and memory. **A–F** The Morris water maze test was used to evaluate spatial learning and memory. **A** Latency times (s) were analyzed for mice subjected to 6 trials/day. **B** and **C** Swim speed (cm/s) and total distances traveled (m) were analyzed during acquisition training ($n=8$ –14 mice/group). **D–F** Number of target zone crossings and times spent in each quadrant (s) and the target zone (s) were analyzed during the probe trial ($n=5$ –6 mice/group). Data are presented as means $\pm$ SEMs. No significant difference was found between vehicle and curcumin-treated mice. **G** The passive avoidance test showed that mice administered curcumin at 0.4 mg/kg daily (the low-dose group) had better memory retention than mice in the vehicle group 7 days after training ($n=8$ mice/group). Data are presented as means $\pm$ SEMs. * $p < 0.05$ vs. Vehicle

RNA silencing

NF1X was knocked down in C17.2 NPCs using siRNA (sequence 5'-TTGGCNNNNNGCCAA-3') purchased from Origene Technologies (Rockville, MD). To select the most efficient siRNA, C17.2 NPCs were transfected with siRNAs using Lipofectamine® 2000 (Invitrogen Life Technologies), according to the manufacturer's instructions. Briefly, C17.2 NPCs were seeded without antibiotics at 5×10^3 cells/well in a 96 well plate. After washing with PBS, cells were transfected for 12 h. Cell viabilities after treatment with curcumin (500 nM) or vehicle were determined using an MTT assay.

Statistical analysis

Analysis of variance (ANOVA) and t-test were used to determine the significance of differences between groups, and post hoc tests were performed to compare groups using Fisher's protected least significant difference (PLSD) procedure. The analysis was performed using Statview software® (Version 5.0.1., SAS Institute Inc., Cary, NC), and p values < 0.05 were considered significant.

Results

Low-dose curcumin increased numbers of newly generated cells in the DG

In order to determine the effect of curcumin on the proliferation and survival of NPCs, 5-week-old C57BL/6 mice were administered curcumin (0.4, 2, or 10 mg/kg, orally) or vehicle for 14 days (Fig. 1B). To label new born cells in the hippocampus (proliferation study), six doses of BrdU were administered i.p. for three consecutive days starting on the 12th day of curcumin administration (Fig. 1B). For the survival study, BrdU was given i.p. for 3 days before curcumin

administration (Fig. 1B), and cells that divided during this 3-day period were observed by BrdU immunostaining (see Materials and Methods). Low-dose curcumin (0.4 mg/kg) significantly increased numbers of BrdU-positive cells compared with vehicle-treated mice in both the survival and proliferation studies, but curcumin at 2 or 10 mg/kg had no significant effect (Fig. 2A, B). These observations indicate orally administered curcumin has neurogenic effects on hippocampal neurogenesis at the relatively low dose of 0.4 mg/kg.

To evaluate the effects of curcumin on neuronal differentiation and the survival of newly-generated cells, double label confocal immunohistochemical analysis was performed using antibodies against BrdU and NeuN (the mature neuronal marker). At 14 days after BrdU administration, BrdU-positive cells migrated into the granule cell layer and were co-labeled with NeuN in the DG, indicating newly-generated cells survived to become mature neurons. BrdU-positive cells in the low-dose curcumin group showed a greater degree of NeuN co-labeling, indicating that low-dose curcumin enhanced hippocampal neurogenesis by increasing the numbers of newly generated neurons (Fig. 2C). Notably, the percentage of BrdU- and NeuN-positive co-labeled cells among BrdU positive cells in the DG of low-dose curcumin-treated mice was significantly greater for vehicle-treated mice (Fig. 2D). Furthermore, low-dose curcumin-treated mice showed significantly more DCX-positive cells and higher DCX expression levels in hippocampus than vehicle treated controls (Fig. 2E, F). Thus, low-dose curcumin enhanced neural proliferation, differentiation, and survival and increased hippocampal neurogenesis.

Hippocampal BDNF and pCREB immunoreactivities were increased by low-dose curcumin

Adult hippocampal neurogenesis can be stimulated by several growth factors, such as BDNF, IGF-1, and VEGF (Liu and Nusslock 2018; Littlejohn et al. 2020; Zhu et al. 2020). BDNF expression is regulated by cAMP response element binding (CREB), a known transcription factor of BDNF that is associated with the regulation of neurogenesis (Esvald et al. 2020; Gao et al. 2022). A BDNF ELISA assay and pCREB immunohistochemistry were used to quantify BDNF and pCREB protein levels in the hippocampal tissues of vehicle- or curcumin-treated mice. Low-dose curcumin upregulated hippocampal BDNF levels (Fig. 3A) and pCREB immunoreactivity (Fig. 3B), which is associated with enhanced hippocampal neurogenesis. These findings show low-dose curcumin

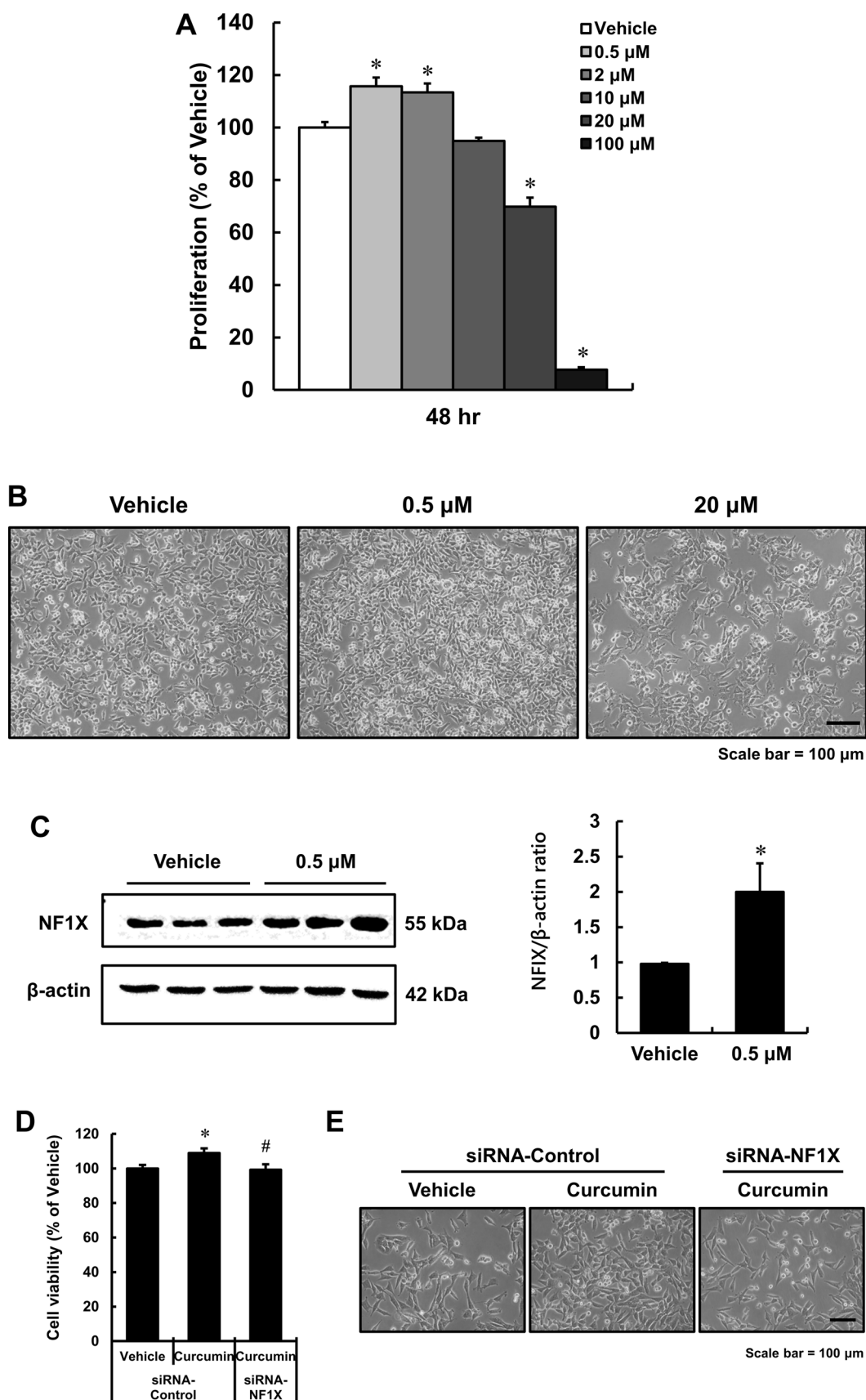


Fig. 5 Low concentration curcumin increased NPC proliferation by upregulating NF1X levels. **A** C17.2 NPCs proliferative abilities after curcumin treatment were evaluated using an MTT assay ($n=8$). Data are presented as means $\pm$ SEMs. $*p<0.05$ vs. Vehicle. Low concentrations of curcumin (0.5 or 2 μ M) increased cell proliferation, but high concentrations (≥ 20 μ M) reduced cell viability after treatment for 48 h. **B** Representative images showing the biphasic effects of curcumin on C17.2 NPCs. Scale bar = 100 μ m. **C** NF1X protein levels in cell lysates were determined by western blot performed independently three times. Data are presented as means $\pm$ SEMs. $*p<0.05$ vs. Vehicle. **D** MTT assays showed that NF1X gene knockdown blocked the proliferative effect of curcumin on C17.2 NPCs. Data are presented as means $\pm$ SEMs. $*p<0.05$ vs. siCon transfected vehicle-treated cells, $\#p<0.05$ vs. siCon transfected curcumin-treated cells. **E** Representative images showing the effects of curcumin after NF1X knockdown in C17.2 NPCs. Scale bar = 100 μ m

promoted hippocampal neurogenesis by upregulating BDNF expression and CREB activity.

Curcumin improved memory retention

Neurocognitive deficits are closely associated with brain aging and neurodegenerative diseases (Gonzales et al. 2022), and it has been established that natural phytochemicals, including curcumin, attenuate cognition and hippocampus-dependent learning and memory deficits associated with neurological disorders and aging (Spencer 2010; Kumar and Khanum 2012). To evaluate whether the neurogenic effects of curcumin influence hippocampus-dependent learning and memory, we subjected C57BL/6 mice to Morris water maze testing to assess spatial learning and memory and passive avoidance test to assess memory retention. Repeated training reduced the latency times of vehicle and curcumin-treated mice in the Morris water maze test. However, no significant difference was observed between the vehicle and curcumin-treated groups in terms of latency times, swim speeds, and total distances traveled (Fig. 4A–C). Twenty-four hours after acquisition training, a probe trial was performed to assess spatial reference memory. Times spent in each quadrant (s), number of target zone crossings, and times spent in the target zone (s) were not different in the vehicle

and curcumin-treated groups (Fig. 4D–F). Interestingly, low-dose curcumin significantly increased step-through latency time in the passive avoidance test 7 days after training, which indicated curcumin improved memory retention (Fig. 4G).

Low concentration curcumin increased the proliferation of C17.2 NPCs by increasing NF1X expression

A previous study demonstrated that curcumin at concentration of 500 nM promotes the proliferation of C17.2 NPCs and NSCs (Kim et al. 2008). In the present study, we observed the effects of curcumin treatment for 48 h on C17.2 NPCs proliferation using an MTT assay. Low concentration of curcumin (0.5 or 2 μ M) increased cell proliferation, whereas high concentrations of curcumin (≥ 20 μ M) decreased cell viability after treatment for 48 h (Fig. 5A). Phase microscopy showed 0.5 μ M curcumin increased cell proliferation and that at 20 μ M curcumin reduced NPCs viability (Fig. 5B), which showed curcumin has biphasic effects on NPCs proliferation.

ACP-based PCR using the GeneFishing™ DEG kit was used to determine how low concentration of curcumin regulated to enhance NPCs proliferation (See supplemental Materials & Methods). Among 20 amplicons displayed, 8 amplicons that were differentially expressed were excised from gels (Fig. S1A). To confirm ACP-based PCR data, we performed direct sequencing to re-amplified expressed band using the GENCLEAN® II Kit (Fig. S1B). Selected differentially expressed amplicons are described in Table 1. We focused on nuclear factor 1/X (NF1X) for DEG 6, which was high expressed by 0.5 μ M curcumin. Western blot confirmed that 0.5 μ M curcumin significantly increased NF1X protein levels in C17.2 NPCs (Fig. 5C). To determine whether NF1X participated in the neurogenic effects of curcumin, we knocked down its expression in C17.2 NPCs using siRNA (Fig. S1C). The mitogenic effects of curcumin were abolished by NF1X siRNA transfection (Fig. 5D,

Table 1 Sequencing results and analysis

DEG No.	Gene symbol	Sequence description	Length
DEG 2	Unknown		348
DEG 3	A130040M12Rik	RIKEN cDNA 2010305A19 gene [<i>Mus musculus</i> (house mouse)]	901
DEG 5	APPL2	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2 [<i>Homo sapiens</i> (human)]	2960
DEG 6	NF1X	nuclear factor 1/X [<i>Mus musculus</i> (house mouse)]	1444

DEG differentially expressed genes

E), indicating that NF1X plays a crucial role in the pathway responsible for the neurogenic effects of curcumin at low concentration.

Discussion

Curcumin has an extensive history as a food preservative and medicinal herb, and it is possible that widespread curcumin consumption might reduce the age-adjusted prevalence of AD (Maiti et al. 2016). Studies have shown curcumin has protective effects in models of irradiation-induced cerebral injury (Xie et al. 2014), cisplatin-induced cognitive impairment (Yi et al. 2020; Babcock et al. 2021), autism (Zhong et al. 2020), traumatic brain injury (Sun et al. 2020), chronic constriction injury (Du et al. 2021), and stroke (Liu et al. 2017). The majority of papers on the ameliorative effects of curcumin on inflammation and injury mechanisms addressed the modulation of NF- κ B or triggering of the Nrf2-ARE and MAPK signaling pathways (Dong et al. 2014; Li et al. 2016; Dai et al. 2018). In a rat model, chronic treatment with curcumin attenuated colchicine-induced cognitive impairment (Kumar et al. 2007), and in another, the antioxidant effect of curcumin improved 3NP-induced motor dysfunction and cognitive deficit (Kumar et al. 2007). In a previous study, we reported that acute administration of curcumin (500 nmole/kg) enhanced hippocampal neurogenesis in adult mice (Kim et al. 2008), and in the present study, sub-chronic oral treatment with low-dose curcumin increased BrdU-positive cells in both proliferation and survival studies, which demonstrated the neurogenic effect of curcumin on hippocampal neurogenesis.

However, higher doses of curcumin (2 or 10 mg/kg) did not increase numbers of newly generated cells in hippocampus, indicating curcumin has a biphasic effect on hippocampal neurogenesis. Others have reported that vitamins and several phytochemicals have biphasic dose-dependent effects on cells and organs, which called the hormesis (Mattson 2008; Jodynis-Liebert and Kujawska 2020; Rai et al. 2021; Sahebnaasagh et al. 2022). In the toxicology field, hormesis is used to explain why phytochemicals have beneficial properties at low-doses but harmful effects at high-doses. Low-doses of phytochemicals expose cells or organs to mild stresses that lead to the activations of cell-survival signaling pathways, such as the MAPK signaling pathway and the transcription factors Nrf2 and CREB (West et al. 2001; Jeong et al. 2006; Lee et al. 2014).

Previous studies have concluded that increased levels of BDNF are associated with the amelioration of learning and memory deficits in rodent models of AD or traumatic brain

injury (Braschi et al. 2021; Gustafsson et al. 2021). BDNF promotes hippocampal neurogenesis and neuronal plasticity, and its expression is regulated by the phosphorylation of CREB at Ser 133 (Numakawa et al. 2017; Wang et al. 2018). Curcumin was reported to have a protective effect on D-galactose-induced cognitive impairment and cadmium-induced neurotoxicity by upregulating BDNF and CREB signaling (Nam et al. 2014; Namgyal et al. 2020), and in the present study, low-dose curcumin increased BDNF and pCREB levels in hippocampal DGs. These observations mirror low-dose curcumin-induced enhancement of neurogenesis in hippocampus. Cognitive function is positively correlated with hippocampal neurogenesis, and it has been suggested that neurogenesis is required for certain types of learning and memory (Kim et al. 2012). Furthermore, it was also reported that chronic administration of curcumin could ameliorate age-related spatial memory deficits (Conboy et al. 2009). In the current study, low-dose curcumin effectively increased memory retention in the passive avoidance test (Fig. 4B) but had no effect on learning and memory as tested by Morris water maze testing (Fig. 4A), which suggests curcumin enhances the contribution of neurogenesis to memory retention in the hippocampus.

The direct molecular targets of low-dose curcumin have not been elucidated. However, in a previous study, we found at low concentration (0.5 μ M) curcumin stimulated the proliferation of C17.2 NPCs and neural stem cells by activating ERK and p38 kinases (Kim et al. 2008). Several studies have demonstrated that curcumin has neurogenic effects. Heidari et al. investigated the neurogenic and neuroprotective effects of curcumin-induced neural stem proliferation and differentiation in different neurological disease model (Heidari et al. 2020), and others have reported that curcumin enhances hippocampal neurogenesis in backgrounds of traumatic brain injury, cadmium-induced neurotoxicity, and cerebral ischemia (Sun et al. 2020; Namgyal et al. 2020; Yang et al. 2021). It has also been reported that curcumin attenuated arsenic-induced ROS generation in neural stem cells and protected neural stem cells against arsenic-induced apoptosis by reducing the activity of caspase-3 (Jahanbazi Jahan-Abad et al. 2017) and attenuated bisphenol A (BPA)-induced neurogenesis suppression by activating the Wnt/ β -catenin pathway (Tiwari et al. 2016). In this study, we propose that the proliferative ability of curcumin is mediated by NF1X, a member of the nuclear factor one (NF1) family, which consists of NF1A, NF1B, NF1C, and NF1X (Heng et al. 2012).

NF1X is expressed in neural stem cells throughout hippocampal morphogenesis (Heng et al. 2012) and has been reported to play critical roles in the proliferation and migration of neural progenitor cells during the development of the SVZ of lateral ventricles (Heng et al.

2014). NF1X is required for normal brain development and essential for the proliferation of NSCs and NPCs (Campbell et al. 2008; Fane et al. 2017). Interestingly, it has been demonstrated that NF1X participates in neuroblast maturation and adult NSCs survival in the SGZ of hippocampal DG and SVZ of lateral ventricles (Harris et al. 2018; Zalucki et al. 2019). These reports show NF1X is closely associated with the maintenance and differentiation of neural progenitor cells, especially in the adult hippocampus and SVZ.

In conclusion, the current study demonstrates that the oral administration of curcumin has biphasic effects on adult hippocampal neurogenesis and cognitive function. Low-dose curcumin increased adult hippocampal neurogenesis and memory retention by stimulating BDNF-pCREB signaling. Furthermore, our in vitro study revealed that the mechanism responsible for the neurogenic effects of curcumin at low concentration involves transcriptional regulation by NF1X. These findings suggest that curcumin is a potential neurogenic agent that might increase hippocampal neurogenesis and ameliorate cognitive deficits.

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Declarations

Conflict of interest The authors have no potential conflict of interest to declare.

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