Hericium erinaceus Extract Reduces Anxiety and Depressive Behaviors by Promoting Hippocampal Neurogenesis in the Adult Mouse Brain

Article *in* Journal of Medicinal Food · November 2017

DOI: 10.1089/jmf.2017.4006

CITATIONS
34

READS
669

5 authors, including:

Joo Youn Kim
National Evidence Based Healthcare Collaborating Agency
33 PUBLICATIONS **805** CITATIONS
SEE PROFILE

Seong Yun Kim
Catholic University of Korea
77 PUBLICATIONS **1,674** CITATIONS
SEE PROFILE

Kyung-Ok Cho
Catholic University of Korea
62 PUBLICATIONS **1,311** CITATIONS
SEE PROFILE

JOURNAL OF MEDICINAL FOOD
J Med Food 00 (0) 2017, 1–7

DOI: 10.1089/jmf.2017.4006

FULL COMMUNICATION

Hericium erinaceus Extract Reduces Anxiety and Depressive Behaviors by Promoting Hippocampal Neurogenesis in the Adult Mouse Brain

Sun Ryu,[1,*] Hyoun Geun Kim,[1,*] Joo Youn Kim,[2] Seong Yun Kim,[1] and Kyung-Ok Cho[1]

[1]*Department of Pharmacology, Catholic Neuroscience Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea.*
[2]*Division of New Health Technology Assessment, National Evidence-Based Healthcare Collaborating Agency, Seoul, Korea.*

ABSTRACT Versatile biological activities of *Hericium erinaceus* (HE) have been reported in many brain diseases. However, roles of HE in major psychiatric disorders such as depression and anxiety remain to be investigated. Therefore, we evaluated whether HE could reduce anxiety and depressive behaviors in the adult mouse and its underlying mechanisms. Male C57BL/6 mice were administered HE (20 or 60 mg/kg, p.o.) or saline once a day for 4 weeks. Open field and tail suspension tests were performed 30 min after the last administration of HE, followed by forced swim test 2 days later. We found that chronic administration of HE showed anxiolytic and antidepressant-like effects. To elucidate possible mechanisms, proliferative activity of the hippocampal progenitor cells was assessed by immunohistochemistry of proliferating cell nuclear antigen (PCNA) and Ki67. Moreover, to evaluate neuronal survival in the dentate gyrus, 5-bromo-2′-deoxyuridine (BrdU) (120 mg/kg, i.p.) was given at the first day of HE administration, followed by isolation of the brains 4 weeks later. HE (60 mg/kg) increased the number of PCNA- and Ki67-positive cells in the subgranular zone of the hippocampus, indicating increased proliferation of hippocampal progenitors. In addition, BrdU- and BrdU/NeuN-positive cells in the dentate gyrus were significantly increased when treated with HE (60 mg/kg) compared with the saline-treated group, demonstrating enhanced neurogenesis by HE treatment. Taken together, the results indicate that chronic HE administration can exert anxiolytic and antidepressant-like effects, possibly by enhancing adult hippocampal neurogenesis.

KEYWORDS: • *adult neurogenesis* • *antidepressant* • *anxiolysis* • **Hericium erinaceus** • *hippocampus* • *mushroom*

INTRODUCTION

Depression and anxiety disorders are serious and burdensome psychiatric illnesses affecting more than 300 million people worldwide.[1] Environmental, genetic, and epigenetic factors can influence symptoms of anxiety and depression, contributing to the complex pathophysiological mechanisms of mood disorders.[2] Moreover, the heterogeneous nature of the diseases makes it more complicated to identify critical factors for treating mood dysregulation.[2] Supporting this notion, antidepressants used for the treatment of both depression and anxiety disorders could show the amelioration of depressive symptoms in only 30–50% of the patients.[3] Extensive research efforts have been devoted to finding the basic mechanisms explaining the pathophysiology of affective mood disorders.[2] Among them, adult hippocampal neurogenesis has been associated with emotional behaviors, memory, and neurodegenerative diseases. It has been shown that these newborn hippocampal neurons are functionally integrated into the existing neuroanatomical circuitry[4,5] and are positively correlated with the management of stress and altered mood.[6–8] For example, ablation of hippocampal neurogenesis increased anxiety and behavioral despair,[9] whereas enhanced hippocampal neurogenesis reduced anxiety and depression-like behaviors.[10] Therefore, developing drugs or functional foods targeting adult hippocampal neurogenesis may provide an improvement for the treatment of mood disorders.

Hericium erinaceus (HE), a common edible medicinal mushroom known as Lions mane, has been traditionally used to treat diverse human diseases in Asian countries. HE consists of various bioactive compounds including polysaccharides, terpenoids, and lectins, which are known to have neuroprotective, antioxidative, anti-inflammatory, and anticancer effects.[11,12] In particular, erinacine, one of the diterpeniods, is reported to facilitate nerve growth factor (NGF) expression and secretion.[13] Given that NGF plays pleiotropic roles in diverse brain diseases, including Alzheimer's disease,[14] HE could alleviate memory deficits in animal models[15,16] as well as in human patients with Alzheimer's disease.[17] Moreover, HE administration reduced

*These authors contributed equally to this work.

Manuscript received 12 May 2017. Revision accepted 24 September 2017.

Address correspondence to: Kyung-Ok Cho, MD, PhD, Department of Pharmacology, Catholic Neuroscience Institute, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Republic of Korea, E-mail: kocho@catholic.ac.kr

stroke-induced cerebral infarction.[18] *In vitro* studies further demonstrated the efficacy of HE against endoplasmic reticulum stress-induced cell death and excitotoxicity.[19,20] However, despite beneficial roles of HE in many neurodegenerative diseases,[21] there have been few reports examining the effects of HE on psychiatric disorders. Although one clinical study gives us a hint about the antidepressive and anxiolytic effects of HE,[22] it still remains to be elucidated how HE can alter emotional behaviors. In this study, we demonstrate that chronic administration of HE in adult C57BL/6 mice shows antianxiety and antidepressant-like effects and it promotes adult hippocampal neurogenesis.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (8 weeks old; Koatech, Kyungki-do, Korea) were housed at a standard temperature (22°C ± 2°C) in a light-controlled environment (light on from 8:00 AM to 8:00 PM) with free access to food and water. Animal experiments were approved by the Ethics Committee of The Catholic University of Korea (2010-0042-03).

HE administration

HE extracted using 70% ethanol was kindly provided by Dr. Young-Ock Kim at Rural Development Administration in South Korea. The experimental schedules are shown in Figure 1. HE (20 or 60 mg/kg) or saline was orally administered daily for 28 days, which was freshly dissolved in saline every day immediately before administration.

Open field test

The open field test was performed to evaluate general motor activity and anxiety level in mice, as previously described with a slight modification.[23] Mice were placed in a 40 × 40 × 30 cm box with 16 equal squares and were allowed to explore the arena for 5 min (day 1). The open field test was performed 30 min before the first administration of HE or saline (day 1) and 30 min after the last administration of HE extracts (day 28). Data were recorded using a video camera and analyzed by SMART software (PANLAB, Barcelona, Spain). Total number of crossings to adjacent squares and total distance explored were scored to analyze locomotor activity and the time spent in the peripheral area (12 squares around the border line) was collected for assessing anxiety level.

Tail suspension test

Tail suspension test (TST) was carried out in a dark room with minimal background noise at 28 days after chronic HE administration. After completing the open field test, each mouse was suspended by its tail using an adhesive tape (1–1.5 cm from the end of the tail) for 6 min with the head 8 cm from the floor. Experiments were recorded using a digital video camera system. Immobility was defined as passive hanging postures and the failure of struggling motion. The immobility time was measured for the last 4 min of the 6 min session. After the test, mice were returned to their home cage.

Forced swim test

Forced swim test (FST) was performed at 2 days after TST in a dark room with minimal background noise. Mice were placed in a 20 cm diameter × 35 cm height plastic cylinder filled with ambient temperature water (22°C ± 2°C) up to 20 cm height of the cylinder. Using a digital video camera system, immobile behavior was monitored for the last 4 min out of total of the 6 min session. Immobility was defined as the absence of all movements except for the motions required to maintain the animal's head above the water.

5-Bromo-2′-deoxyuridine administration and tissue preparation

Mice received five 5-bromo-2′-deoxyuridine (BrdU) injections (120 mg/kg, i.p.) with the interval of 1 h at day 0. At 2 h after the last BrdU injection or the completion of FST, mice were deeply anesthetized with chloral hydrate (500 mg/kg, i.p.) and transcardially perfused with saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4). Brains were removed and postfixed in paraformaldehyde for 24 h and then dehydrated with 30% sucrose in 0.1 M PB for 3 days. Brains were embedded in Tissue-Tek (Sakura Finetechnical, Tokyo, Japan) and were rapidly frozen with liquid nitrogen. Using a cryostat, 20-μm-thick serial coronal sections were collected at every fifth section with the interval of 80 μm (total 400 μm, between −1.58 and −1.98 from bregma) and were further processed for staining.

Immunohistochemistry

Tissue sections were mounted on the MAS-coated slide glass and were boiled in 0.01 M citrate buffer (pH 6.0) using a microwave for 5 min. After cooling, the sections were blocked with 3% bovine serum albumin and 0.01% Triton X-100 in 0.01 M phosphate-buffered saline (PBS) for 1 h and were incubated overnight at 4°C with primary antibodies to proliferating cell nuclear antigen (PCNA; 1:100; Chemicon Int.,

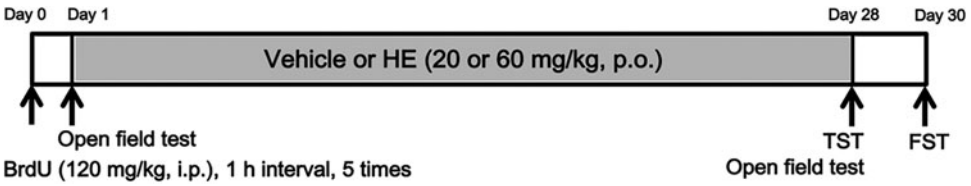

FIG. 1. Schematic diagram for experimental procedures. All experiments were performed on the date indicated. FST, forced swim test; HE, *Hericium erinaceus*; TST, tail suspension test.

Temecuala, CA, USA) or Ki67 (1:100; Novocastra Laboratories Ltd., New castle, United Kingdom). The next day, the sections were incubated with secondary antibodies, cy-3-conjugated IgG for Ki67 and Alexa Fluor 488 (Invitrogen, Eugene, OR, USA)-conjugated rat IgG for PCNA, for 2 h at room temperature. Then the sections were cover slipped with Prolong Gold mounting medium (Invitrogen) and were observed using fluorescent microscopy (Axioimager M1; Carl Zeiss Co., Ltd., Jena, Germany). For double immunofluorescence, free floating sections were incubated in 50% formamide/2 × saline sodium citrate (0.3 M NaCl, 0.03 M sodium citrate) for 2 h at 65°C and further treated with 2 N HCl for 1 h, then with 0.1 M boric acid (pH 8.5) for 10 min at 37°C. After blocking with 10% normal goat serum, the sections were incubated with a primary antibody to BrdU (1:100; DAKO, Glostrup, Denmark) overnight at 4°C. The next day the sections were incubated with cy-3-conjugated mouse monoclonal antibody for 2 h at room temperature. After rinsing with PBS three times, the sections were incubated with the primary antibody to NeuN (1:200; Millipore, Boston, MA, USA) overnight, followed by Alexa Fluor 488-conjugated rabbit IgG for 2 h. Then the sections were mounted and observed using a confocal microscopy (LSM 510 Meta; Carl Zeiss Co., Ltd.).

Cell counting

The number of immunoreactive cells to PCNA, Ki67, BrdU, and BrdU/NeuN was counted in a double-blind manner. The total number of positive cells in the subgranular zone of the dentate gyrus (defined as a 2-cell body width zone along the lower border region of the granule cell layer) on each slide in all animals was counted. When the cells were clustered, each immunoreactive cell was identified by adjusting the focus of the field. The number of immunoreactive cells from each section was all added and used in the statistical analysis.

Statistical analysis

Data were expressed as mean ± SEM and statistical significance was assessed using GraphPad Prism 7 software. Total number of crossings in open field test and TST was analyzed by Kruskal–Wallis nonparametric test followed by Dunn's multiple comparisons test because Gaussian distribution was not assumed. For the rest of the data, one-way analysis of variance (ANOVA) and Dunnett's *post hoc* test were performed. $P < .05$ was considered to be significant.

RESULTS

Effect of chronic HE administration on anxiety

The level of anxiety was evaluated by measuring the time spent in the peripheral zone of the open field box. On the last day of chronic HE administration for 28 days (Fig. 1), vehicle group explored the peripheral zone for 274.10 ± 3.15 s during 5 min of test (Fig. 2A). The HE-treated group with 20 mg/kg stayed in the peripheral zone for 261.10 ± 8.00 s, which was not different from that of the vehicle group. However, the HE-treated group with 60 mg/kg showed a significant reduction in the time spent in the peripheral zone of the open field arena (230.00 ± 15.12 s) compared with vehicle group, suggesting anxiolytic effects of chronic high-dose HE administration.

Effect of chronic HE administration on general locomotion

To rule out a possibility that chronic HE administration could affect general locomotor activity and thereby acting as a confounding bias, the open field test was performed before the initial and the last HE administration (day 0, day 28, respectively). Total number of crossings to the adjacent squares and the total exploratory distance showed no significant differences among the three groups (Fig. 2B, C). These data indicate that chronic HE treatment did not affect general locomotor

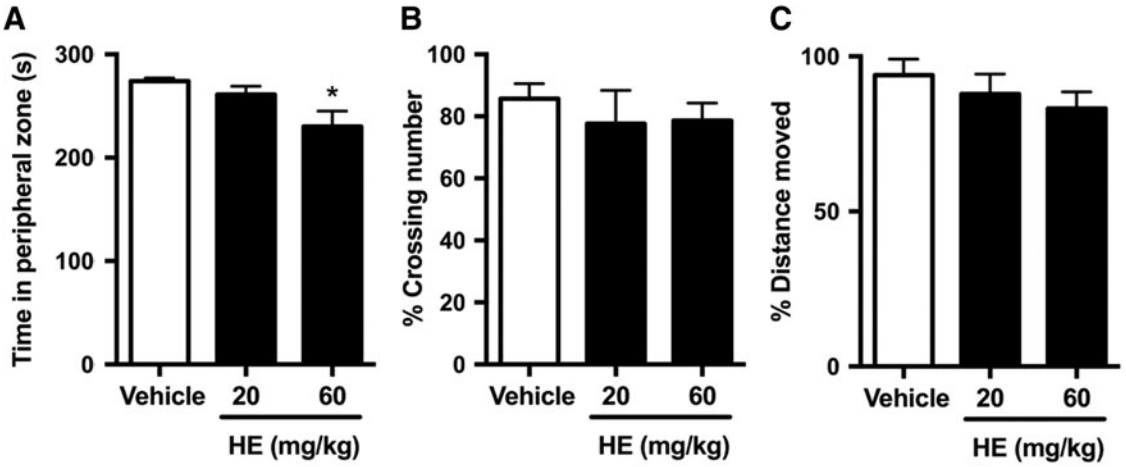

FIG. 2. Effects of chronic HE treatment on anxiety-like behavior in the open field test. A graph showing exploratory time in the peripheral zone of the open field arena **(A)**. The number of crossings to adjacent squares **(B)** and the distance moved **(C)** after chronic HE treatment were divided by the values tested before the initial HE treatment. HE treatment did not affect locomotion in the open field test. Data are expressed as mean ± SEM. *$P < .05$ vs. vehicle.

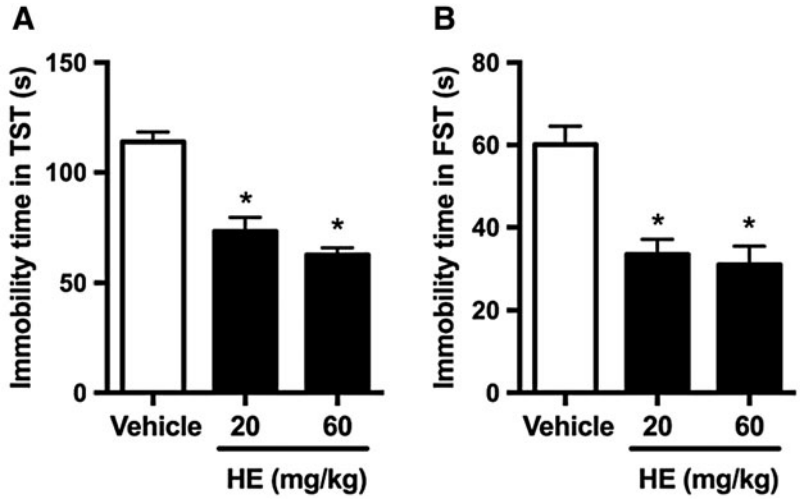

FIG. 3. Effects of chronic HE treatment on depressive behavior. TST **(A)** and FST **(B)** showed that chronic HE treatment significantly shortened the duration of immobility, which is indicative of antidepressant-like behavior. Data are expressed as mean ± SEM.*$P < .05$ vs. vehicle.

function in mice, suggesting that the effect was attributable to psychiatric beneficial effects after HE administration.

Effect of chronic HE administration on depression-like behavior

TST and FST were chosen to assess the effects of HE treatment on depression. For sham mice, the time spent immobile in TST was 114.00 ± 4.47 s during 4 min of test. However, the immobility time was significantly decreased in the group with 60 mg/kg of HE administration, at 62.40 ± 3.25 s (Fig. 3A). In FST, the immobility time for the HE-treated group (60 mg/kg) was also significantly reduced to 31.12 ± 4.29 s compared with the sham group wherein the immobile duration was 60.07 ± 4.53 s (Fig. 3B). Interestingly, the immobility time for the group with 20 mg/kg of HE administration was 73.16 ± 6.37 s in TST and 33.45 ± 3.65 s in FST, which were significantly decreased compared with those in vehicle group (Fig. 3). These data demonstrate antidepressant-like effects by chronic HE administration in adult mice.

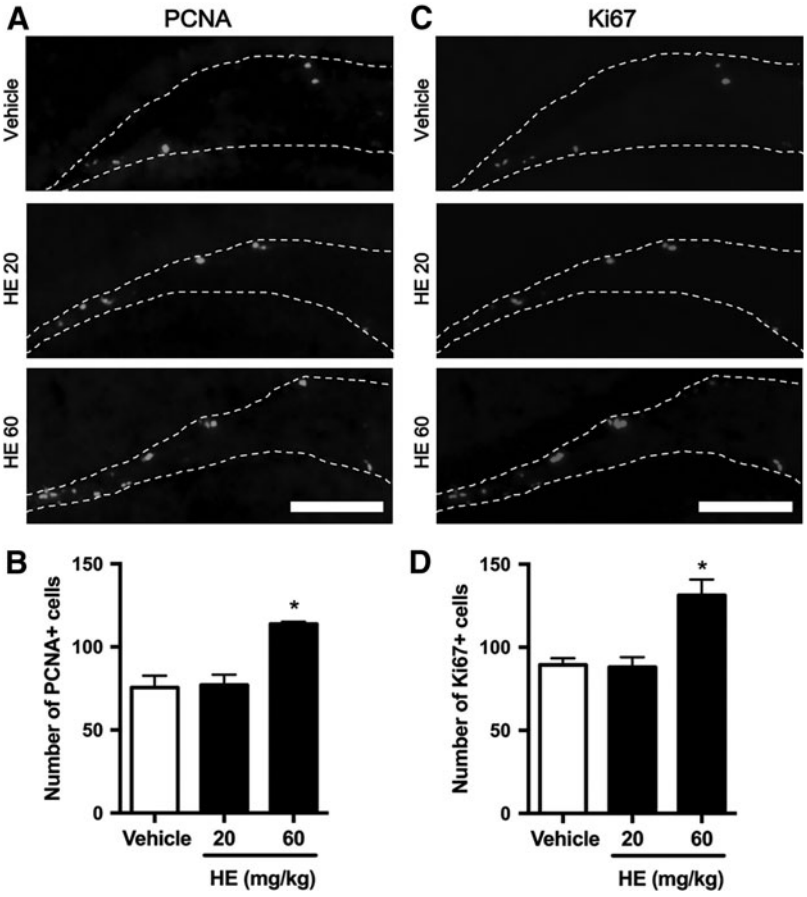

FIG. 4. Effects of chronic HE treatment on the proliferative activity of the hippocampus. Representative images of cells expressing PCNA **(A)** and Ki67 **(C)** in the dentate gyrus. The number of cells expressing PCNA **(B)** and Ki67 **(D)** in the subgranular zone was significantly increased in 60 mg/kg of HE group compared with vehicle group. Data are expressed as mean ± SEM. *$P < .05$ vs. vehicle. A scale bar in **(A)** and **(C)** is 100 μm. PCNA, proliferating cell nuclear antigen.

Proliferative activity in the hippocampus by chronic HE administration

To determine the mechanisms of anxiolytic and antidepressant-like effects of chronic HE administration, proliferative activity of the hippocampal stem/progenitor cells was assessed. As shown in Figure 4A, B, the number of PCNA-immunoreactive cells in the subgranular zone was significantly increased in the HE group with 60 mg/kg (113.80 ± 1.38) compared with sham mice (75.60 ± 7.08). In addition, the number of Ki67-expressing cells in the subgranular zone was increased in the HE group with 60 mg/kg (131.30 ± 9.48) compared with vehicle group (89.40 ± 4.03), consistent with the PCNA results (Fig. 4C, D). These results demonstrate that chronic administration of high-dose HE significantly enhances proliferative activity of the hippocampal stem/progenitor cells.

Effect of chronic HE administration on hippocampal neurogenesis

To further elucidate the role of HE administration in hippocampal neurogenesis, survival of both the newborn cells and the newly generated neurons was assessed by BrdU and BrdU/NeuN immunofluorescence, respectively. As shown in Figure 5, BrdU-positive cells in the granule cell layer of the dentate gyrus were significantly increased after chronic administration of 60 mg/kg of HE (51.56 ± 3.64) compared with those in the sham group (31.83 ± 1.86). Moreover, BrdU/NeuN-double positive cells, which indicate newly born cells that were present right before the first HE administration and were differentiated to mature neurons during HE treatment, were significantly increased after chronic high-dose administration of HE (48.78 ± 3.55 in 60 mg/kg, 28.50 ± 1.26 in vehicle). To estimate the number of newly born cells before the first HE administration, we injected BrdU five times at day 0 and perfused the mice at 2 h after the last BrdU injection. Approximately 26.19% ± 1.16% of BrdU-labeled cells at day 0 were able to survive and become hippocampal neurons, which was also increased by chronic 60 mg/kg of HE treatment (44.83% ± 3.26%). Interestingly, the ratio of BrdU/NeuN–double positive cells based on total BrdU-positive cells at day 30 was similar in all groups (89.5% for vehicle group, 89.9% for 20 mg/kg of HE group, and 94.6% for 60 mg/kg of HE group, respectively), suggesting that neuronal differentiation from hippocampal stem cells was not altered by HE

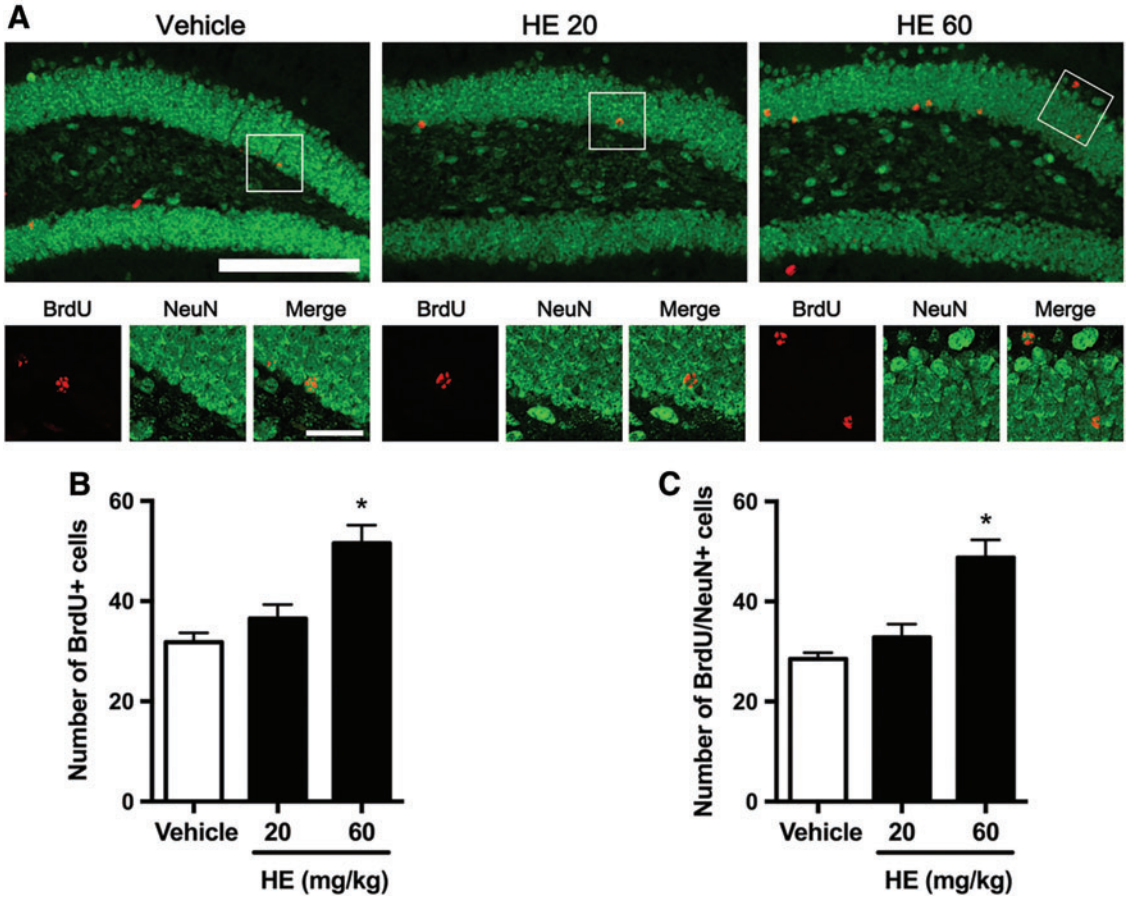

FIG. 5. Effects of chronic HE treatment on hippocampal neurogenesis. Representative images showing immunostaining for BrdU (*red*), NeuN (*green*), BrdU/NeuN double-positive cells in the dentate gyrus of HE- and vehicle-treated mice **(A)**. Magnified images indicate exemplary immunoreactive cells in the dentate gyrus. Cells expressing BrdU **(B)** and BrdU/NeuN **(C)** in the dentate gyrus were significantly increased after chronic administration of 60 mg/kg of HE compared with vehicle group. Data are expressed as mean ± SEM. *$P < .05$ vs. vehicle. Scale bars in **(A)** represent 200 μm (low magnification) and 20 μm (high magnification). Color images available online at www.liebertpub.com/jmf

administration. Taken together, these data demonstrate that chronic high-dose treatment of HE markedly increased the survival of newborn neurons in the dentate gyrus.

DISCUSSION

This study demonstrates that HE treatment for 28 days significantly reduced anxious and depressive behaviors with no difference in general locomotor activity. Moreover, we showed that chronic high-dose HE treatment significantly increased proliferation and survival of hippocampal progenitor cells without altering the ratio of neuronal differentiation, providing a mechanistic link of HE treatment to antianxiety and antidepression.

The edible mushroom HE has been traditionally used as a culinary food and herbal medicine in Asia. Herbal medicines can be attractive alternatives for treating psychiatric disorders as they can provide efficacy similar to conventional drugs such as benzodiazepines or selective serotonin reuptake inhibitors, but they can have less toxic side effects.[12] Indeed, we found chronic HE treatment significantly decreased immobile time when the mice were tested by TST and FST, the two most frequently used behavioral paradigms for evaluating depression in rodents.[24,25] We also showed that 60 mg/kg of HE reduced the time staying at the peripheral zone of the open field arena, indicating anxiolytic effect of HE. Importantly, our chronic HE administration did not affect animals' natural body weight gain (data not shown), suggesting no severe oral gavage-associated stress nor serious toxicity by chronic HE treatment. It is noteworthy that our dosage regimen for HE was in the clinically applicable range as a human study provided 0.5 g of HE powder four times a day to middle-aged females with a body mass index of $20.9 \pm 20.6\,kg/m^2$, resulting in $\sim$40 mg/kg of HE if the average height of the participants was estimated to be 160 cm.[22]

Hippocampal neurogenesis and its perturbation have been implicated as one of the major players in mood dysregulation such as depression and anxiety.[8–10,26–30] In this study, we identified a novel candidate targeting adult hippocampal neurogenesis. Specifically, chronic oral administration of HE (60 mg/kg) for 4 weeks increased the number of proliferating cells in the subgranular zone of the dentate gyrus where neural stem cells reside. Increased production of newborn cells subsequently led to a higher increment of newborn neurons showing increased survival rates, when assessed by BrdU/NeuN immunostaining. Molecular mechanisms underlying HE-enhanced hippocampal neurogenesis may be associated with NGF production, a well-known regulator of the proliferation and the differentiation of neural stem cells.[31,32] Moreover, an *in vitro* study demonstrated that crude HE extracts promoted synthesis and secretion of NGF in astrocytoma cell lines, supporting HE-induced NGF production.[33] In addition, chronic *in vivo* administration of HE extracts increased the level of both NGF mRNA and proteins in the hippocampus, suggesting successful transfer of HE across the blood–brain barrier.[18,33] When narrowed down to active ingredients of HE in relation to NGF, the majority of studies focused on erinacines and hericenones, showing improved neurite outgrowth.[11–13,15,20] Taken together, NGF, possibly stimulated by crude HE extracts that contain erinacines and hericenones, can boost adult hippocampal neurogenesis. However, as many new bioactive compounds comprising HE are actively being identified,[34,35] it will be interesting to find out whether novel molecules are present in the extract that mediates adult hippocampal neurogenesis after chronic HE administration.

Antidepressants often show their efficacy after chronic treatment.[3,4,6] This observation supported the neurogenic theory of mood disorders, which states that symptoms of depression and anxiety are adult neurogenesis dependent.[36] Here we demonstrate that HE has neurogenic and antidepressant-like effects after chronic treatment for 28 days. Given that hippocampal progenitors take at least 1 month before they become mature neurons and single HE treatment does not affect NGF production that is frequently reduced in stress,[18,37–39] antianxiety and antidepressant-like effects by HE administration may need a chronic time frame for allowing the maturation of neurons born during the HE treatment. A clinical study showing reduced depression and anxiety after 4 weeks of HE treatment nicely supports the requirement of chronic HE treatment for mood alteration.[22] However, since we administered crude extracts of HE, there can be other mechanisms for mood-stabilizing effects of HE in addition to the enhancement of hippocampal neurogenesis.

In conclusion, this study is the first to demonstrate that chronic HE administration can promote hippocampal neurogenesis accompanied by mood-modifying effects. Behavioral improvement observed after chronic HE administration in our study endorses the neurogenic theory of mood disorders, as this time frame corresponds to the maturation and functional integration of newborn neurons into the hippocampal circuitry after HE-boosted proliferation of progenitor cells. These results also highlight the potential for the modulation of the brain plasticity and behavior by HE administration.

ACKNOWLEDGMENTS

We thank Dr. Young-Ock Kim and Dr. Sang-Won Lee at Rural Development Administration for the supply of HE extracts. This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean Government (NRF-2014R1A1A3049456), the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare (HI15C2854), and Catholic Medical Center Research Foundation made in the program year of 2015.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. World Health Organization: Depression. 2017. www.who.int/mediacentre/factsheets/fs369/en (accessed February 2017).
2. Krishnan V, Nestler EJ: The molecular neurobiology of depression. *Nature* 2008;455:894–902.

3. Gaynes BN, Rush AJ, Trivedi MH, Wisniewski SR, Spencer D, Fava M: The STAR*D study: Treating depression in the real world. *Cleve Clin J Med* 2008;75:57–66.
4. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH: Functional neurogenesis in the adult hippocampus. *Nature* 2002;415:1030–1034.
5. Shors TJ: Memory traces of trace memories: Neurogenesis, synaptogenesis and awareness. *Trends Neurosci* 2004;27:250–256.
6. Malberg JE: Implications of adult hippocampal neurogenesis in antidepressant action. *J Psychiatry Neurosci* 2004;29:196–205.
7. Kheirbek MA, Klemenhagen KC, Sahay A, Hen R: Neurogenesis and generalization: A new approach to stratify and treat anxiety disorders. *Nat Neurosci* 2012;15:1613–1620.
8. Sahay A, Hen R: Adult hippocampal neurogenesis in depression. *Nat Neurosci* 2007;10:1110–1115.
9. Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA: Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* 2011;476:458–461.
10. Hill AS, Sahay A, Hen R: Increasing adult hippocampal neurogenesis is sufficient to reduce anxiety and depression-like behaviors. *Neuropsychopharmacology* 2015;40:2368–2378.
11. Friedman M: Chemistry, nutrition, and health-promoting properties of *Hericium erinaceus* (Lion's mane) mushroom fruiting bodies and mycelia and their bioactive compounds. *J Agric Food Chem* 2015;63:7108–7123.
12. Phan CW, David P, Sabaratnam V: Edible and medicinal mushrooms: Emerging brain food for the mitigation of neurodegenerative diseases. *J Med Food* 2017;20:1–10.
13. Shimbo M, Kawagishi H, Yokogoshi H: Erinacine A increases catecholamine and nerve growth factor content in the central nervous system of rats. *Nutr Res* 2005;25:617–623.
14. Xu CJ, Wang JL, Jin WL: The emerging therapeutic role of NGF in Alzheimer's disease. *Neurochem Res* 2016;41:1211–1218.
15. Mori K, Obara Y, Moriya T, Inatomi S, Nakahata N: Effects of *Hericium erinaceus* on amyloid beta (25–35) peptide-induced learning and memory deficits in mice. *Biomed Res* 2011;32:67–72.
16. Zhang J, An S, Hu W, *et al.*: The neuroprotective properties of *Hericium erinaceus* in glutamate-damaged differentiated PC12 cells and an Alzheimer's disease mouse model. *Int J Mol Sci* 2016;17:pii:E1810.
17. Mori K, Inatomi S, Ouchi K, Azumi Y, Tuchida T: Improving effects of the mushroom Yamabushitake (*Hericium erinaceus*) on mild cognitive impairment: A double-blind placebo-controlled clinical trial. *Phytother Res* 2009;23:367–372.
18. Hazekawa M, Kataoka A, Hayakawa K, *et al.*: Neuroprotective effect of repeated treatment with *Hericium erinaceum* in mice subjected to middle cerebral artery occlusion. *J Health Sci* 2010;56:296–303.
19. Nagai K, Chiba A, Nishino T, Kubota T, Kawagishi H: Dilinoleoyl-phosphatidylethanolamine from *Hericium erinaceum* protects against ER stress-dependent Neuro2a cell death via protein kinase C pathway. *J Nutr Biochem* 2006;17:525–530.
20. Chang CH, Chen Y, Yew XX, *et al.*: Improvement of erinacine A productivity in *Hericium erinaceus* mycelia and its neuroprotective bioactivity against the glutamate-insulted apoptosis. *LWT Food Sci Technol* 2016;65:1100–1108.
21. Phan CW, David P, Naidu M, Wong KH, Sabaratnam V: Therapeutic potential of culinary-medicinal mushrooms for the management of neurodegenerative diseases: Diversity, metabolite, and mechanism. *Crit Rev Biotechnol* 2015;35:355–368.
22. Nagano M, Shimizu K, Kondo R, *et al.*: Reduction of depression and anxiety by 4 weeks *Hericium erinaceus* intake. *Biomed Res* 2010;31:231–237.
23. Prut L, Belzung C: The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *Eur J Pharmacol* 2003;463:3–33.
24. Porsolt RD, Le Pichon M, Jalfre M: Depression: A new animal model sensitive to antidepressant treatments. *Nature* 1977;266:730–732.
25. Steru L, Chermat R, Thierry B, Simon P: The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology (Berl)* 1985;85:367–370.
26. Jayatissa MN, Bisgaard C, Tingstrom A, Papp M, Wiborg O: Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology* 2006;31:2395–2404.
27. Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K: High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. *Science* 2007;317:819–823.
28. Banasr M, Soumier A, Hery M, Mocaer E, Daszuta A: Agomelatine, a new antidepressant, induces regional changes in hippocampal neurogenesis. *Biol Psychiatry* 2006;59:1087–1096.
29. Oomen CA, Soeters H, Audureau N, *et al.*: Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. *J Neurosci* 2010;30:6635–6645.
30. Revest JM, Dupret D, Koehl M, *et al.*: Adult hippocampal neurogenesis is involved in anxiety-related behaviors. *Mol Psychiatry* 2009;14:959–967.
31. Cattaneo E, McKay R: Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor. *Nature* 1990;347:762–765.
32. Frielingsdorf H, Simpson DR, Thal LJ, Pizzo DP: Nerve growth factor promotes survival of new neurons in the adult hippocampus. *Neurobiol Dis* 2007;26:47–55.
33. Mori K, Obara Y, Hirota M, *et al.*: Nerve growth factor-inducing activity of *Hericium erinaceus* in 1321N1 human astrocytoma cells. *Biol Pharm Bull* 2008;31:1727–1732.
34. Zhang CC, Yin X, Cao CY, Wei J, Zhang Q, Gao JM: Chemical constituents from *Hericium erinaceus* and their ability to stimulate NGF-mediated neurite outgrowth on PC12 cells. *Bioorg Med Chem Lett* 2015;25:5078–5082.
35. Wang XL, Gao J, Li J, *et al.*: Three new isobenzofuranone derivatives from the fruiting bodies of *Hericium erinaceus*. *J Asian Nat Prod Res* 2017;19:134–139.
36. Miller EJ, Saint Marie LR, Breier MR, Swerdlow NR: Pathways from the ventral hippocampus and caudal amygdala to forebrain regions that regulate sensorimotor gating in the rat. *Neuroscience* 2010;165:601–611.
37. Goncalves JT, Schafer ST, Gage FH: Adult neurogenesis in the hippocampus: From stem cells to behavior. *Cell* 2016;167:897–914.
38. Alfonso J, Frick LR, Silberman DM, Palumbo ML, Genaro AM, Frasch AC: Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. *Biol Psychiatry* 2006;59:244–251.
39. von Richthofen S, Lang UE, Hellweg R: Effects of different kinds of acute stress on nerve growth factor content in rat brain. *Brain Res* 2003;987:207–213.