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Influences of Postnatal *Ginseng radix* Administration on Prenatal Noise Stress-induced c-Fos Expression in the Hippocampus of Offspring Rats

Jae chan Jang, Youn Sub Kim, Ji Hyouck Kim*
Department of Anatomy-Pointlogy College of Oriental Medicine, Kyungwon University,
*Raphakyunghee oriental clinic

태아 중 소음 스트레스가 출생 후 해마 내 c-Fos 발현에 미치는 인삼의 효과

장재찬, 김연섭 김지혁*
경원대학교 한의과대학 해부경혈학교실, *라파경희한의원

국문초록

목적 :임신 중 소음 스트레스가 태아에 어떤 영향을 미치는 가를 관찰하고자, 임신 중인 흰쥐에 소음 스트레스를 가한 후 출생한 새끼 흰쥐에 인삼을 투여하여 신경적 발육에 대한, 특히 새끼 흰쥐 해마의 c-Fos 관점에서 연구하였다.

결과 :CIA 영역의 c-Fos-positive 세포의 수는, 출생 전 소음 스트레스를 받은 대조군에 비해, 10 mg/kg의 인삼을 투여한 군에서 유의성 있는 감소를 보였다.

해마의 CA2, CA3 영역에서의 c-Fos-positive 세포의 수는 10 mg/kg의 인삼을 투여한 그룹에서, 출생 전 소음 스트레스를 받은 대조군에 비해 유의성 있는 감소를 보였다.

해마의 dentate gyrus 영역에서의 c-Fos-positive 세포의 수는, 10 mg/kg의 인삼을 투여한 그룹에서, 출생 전 소음 스트레스를 받은 대조군에 비해 유의성 있는 감소를 보였다.

결론: 인삼은 태아기 소음 스트레스로 야기되는 c-Fos의 변화로 인한 해마의 기능장애에 중화제로서 사용될 수 있다. 그리고 임신 중 소음 스트레스를 경험한 엄마의 아이들에 있어서 정신과적 문제를 치료하는데 유용할 것이다.

키워드 : 임신 중 소음스트레스, 인삼, 해마, c-Fos 발현

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* 교신저자 : 김연섭, 경기도 성남시 수정구 복정동 산 65, 경원대학교 한의과대학
E-mail : ysk@kyungwon.ac.kr Tel ; 031-750-5420

Ⅰ. INTRODUCTION

Stress has become an integral part of human life in this modern era. Among the innumerable stressors to which mankind is exposed, noise happens to be a commonly encountered stressor throughout the world. Even brief noise exposure is known to increase heart rate and peripheral vascular resistance, leading to a rise in blood pressure. On the other hand, experimental studies have demonstrated ultra structural modifications in rat cardiomyocytes mainly involving mitochondria. These subcellular alterations are related to an imbalance in calcium homeostasis, which is supposed to be sustained by increased catecholamine innervations.[1,2)]

Noise as a stressful stimulus is a widely accepted fact. However an effective agent to counter the noise stress-induced biochemical alterations remains elusive. *Ginseng radix*, the root of *Panax ginseng*C.A.Meyer (Araliaceae), is one of the most famous Oriental medical herbs and has several therapeutic applications. It is well documented that *Ginseng radix* possesses a number of pharmacological effects including hypotensive, cardiotonic, sedative, aphrodisiac, antiaging, and antioxidant actions.[3,4)]

It was suggested that exposure to prenatal stress alters an individual's developmental trajectory through altered early brain development. In late gestation, the fetus can hear sound from the outside of the mother.[5)] Previous studies reported that exposure to noise during pregnancy adversely influenced the development of the fetus and neonate: increased antepartum fetal death and congenital anomaly in the central nervous system, impaired social behavior in juvenile stage, and a long-term alteration in the immune function[6,7)]. In addition, prenatal noise stressis known to influence learning and memory capabilities of the offspring by altering neuronal activity in the hippocampus.[8)]

Hippocampal formation is a brain region critically involved in learning and memory formation.

In humans, hippocampal damage impairs explicit memory, and in rodents, hippocampal damage impairs spatial and contextual learning which require the formation of relational representations among multiple cues.[9-11)]

c-Fos is an immediate early gene whose expression is sometimes used as a marker for stimulus-induced changes in the metabolic activity of neurons, being induced in the CNS under various conditions.[12-14)] Recently, it was reported that prenatal stress produces region-selective changes in expression of inducible transcription factors (ITFs) and metabolism in the brain.[15,16)] In the present study, the influence of postnatal *Ginseng radix* administration on neuronal changes, in particular with respect to c-Fos expression in the hippocampus of offspring rats with prenatal noise stress during pregnancy was investigated *via* c-Fos immunohistochemistry.

Ⅱ. MATERIALS AND METHODS

1. Animals and treatments

The experimental procedures were performed in accordance with the guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. Male Sprague-Dawley rats (250 ± 10 g, 12 weeks old) and female Sprague-Dawley rats (180 ±10 g, 8 weeks old) were used in this study. Female rats (n = 20) were allowed to mate with male rats (n = 20) for 24 h. One day later, female rats were separated from the male rats and housed individually in a plastic home cage at the controlled temperature (20 ± 2 ℃) and the light-dark cycle of 12 h of light and 12 h of darkness (light on from 07:00 h to 19:00 h). Food and water were made available *ad libitum*. After confirming of pregnancy on the 14 days after mating, female rats were randomly divided into four groups: the control group, the 10 mg/kg *Ginseng radix*-treated group, the noise-treated group, the noise- and 10 mg/kg *Ginseng radix*-treated group (n = 5 for each group). Starting on the 15th day of pregnancy, rats of the prenatal noise-treated groups were applied with the 95 decibel supersonic machine sound for 1 honce a day until delivery.[17] After birth, the offspring in each group was left undisturbed together with the respective mother for 28 days, and then, offspring rats were administered per os (P.O.) with *Ginseng radix* at the respective dose once a day for 7 days; they were sacrificed 6 weeks after birth. To obtain the aqueous extract of *Ginseng radix*, 200 g of *Ginseng radix* was added to distilled water, and extraction was performed by heating at 80℃ concentrated with a rotary evaporator, and lyophilized. The resulting powder, weighing 30 g (a collection rate of 15 %), was diluted with saline.

2. Tissue preparation

For the sacrificial process, animals were first weighed and overdosed with Zoletil 50 (10 mg/kg, i.p.; Vibac, Carros, France). After a complete lack of response was observed, the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS) and then with 4% paraformaldehyde in 100 mM phosphate buffer (PB) at pH 7.4. The brains were dissected, postfixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Serial coronal sections of 40 μm thickness were made using a freezing microtome (Leica, Nussloch, Germany).

3. c-Fos immunohistocheistry

c-Fos immunostaining was performed according to a protocol described by He et al.[18]. Eight sections on average were selected from each brain region spanning from Bregma -3.30 mm to -4.16 mm. Free-floating tissue sections were incubated overnight with rabbit anti-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:1000, and the sections were then incubated for 1 h with biotinylated anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA,

USA) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3-diaminobenzidine and 0.01% H2O2 in 50 mM Tris-buffer (pH 7.6) for approximately 3 min. As the negative control, brain sections from the experiment were likewise processed using normal goat serum as the primary antibody; no c-Fos-like immunoreactivity was observed.

4. Data analysis

To quantify the number of Fos-positive cells in each areas of the hippocampus, cell counting was performed through a light microscope (Olympus, Tokyo, Japan). The number of Fos-positive cells inside pyramidal cell layer was counted hemilaterally in each of the selected hippocampal regions.

5. Statistical analysis

Statistical significance of differences were determined by one-way analysis of variance (ANOVA) followed by Dunncan's post-hoc analysis, and results were expressed as mean ±standard error mean (S.E.M.) of Fos-positive cells. Differences were considered significant for $P < 0.05$.

Ⅲ. RESULTS

1. Number of c-Fos-positive cells in the CA1 region of hippocampus

The number of c-Fos-positive cells in the CA1 region of hippocampus was 50.00 ± 5.56/mm^2 in the control group, 47.06 ± 5.81/mm^2 in the 10 mg/kg *Ginseng radix*-treated group, 143.14 ± 14.63 in the prenatal noise stress-treated group, and 59.80 ± 2.71/mm^2 in the prenatal noise stress- and 10 mg/kg *Ginseng radix*-treated group. The number of prenatal noise stress-treated group was increased significantly compared to the control group. The number of 10 mg/kg *Ginseng radix*-treated groupwas not increased significantly compared to the control group. And, the number of the prenatal noise stress- and 10 mg/kg *Ginseng radix*-treated groupwas decreased significantly compared to the prenatal noise stress-treated group (Fig. 1-2).

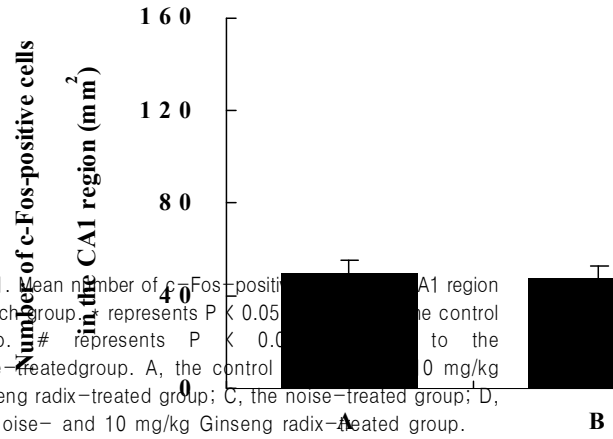


Fig. 1. Mean number of c-Fos-positi A1 region in each group. * represents P < 0.05 e control group. # represents P < 0.0 to the noise-treatedgroup. A, the control 0 mg/kg Ginseng radix-treated group; C, the noise-treated group; D, the noise- and 10 mg/kg Ginseng radix-treated group.



Fig. 2. Photomicrographs of Fos-positive cells in the CA1 region in each group.Sections were stained for c-Fos-positive cells (reddish brown). Scale bar represents 100 um. A, the control group B, the 10 mg/kg Ginseng radix-treated group; C, the noise-treated group; D, the noise- and 10 mg/kg Ginseng radix-treated group.

2. Number of c-Fos-positive cells in the CA2 and CA3 regions of hippocampus

The number of c-Fos-positive cellsin the CA2 and CA3 regions of hippocampus was 11.60 ±0.72/mm^{2}in the control group, 30.00 ± 3.88/mm^2 in the 10 mg/kg *Ginseng radix*-treated group, 56.00 ± 3.43 in the prenatal noise stress-treated group, and 23.20 ± 4.98/mm^2 in the prenatal noise stress- and 10 mg/kg *Ginseng radix*-treated group. The number of prenatal noise stress-treated group was increased significantly compared to the control group. The number of 10 mg/kg *Ginseng radix*-treated group was not increased significantly compared to the control group. And, the number of the prenatal noise stress- and 10 mg/kg *Ginseng radix*-treated groupwas decreased significantly compared to the prenatal noise stress-treated group (Fig. 3-4).

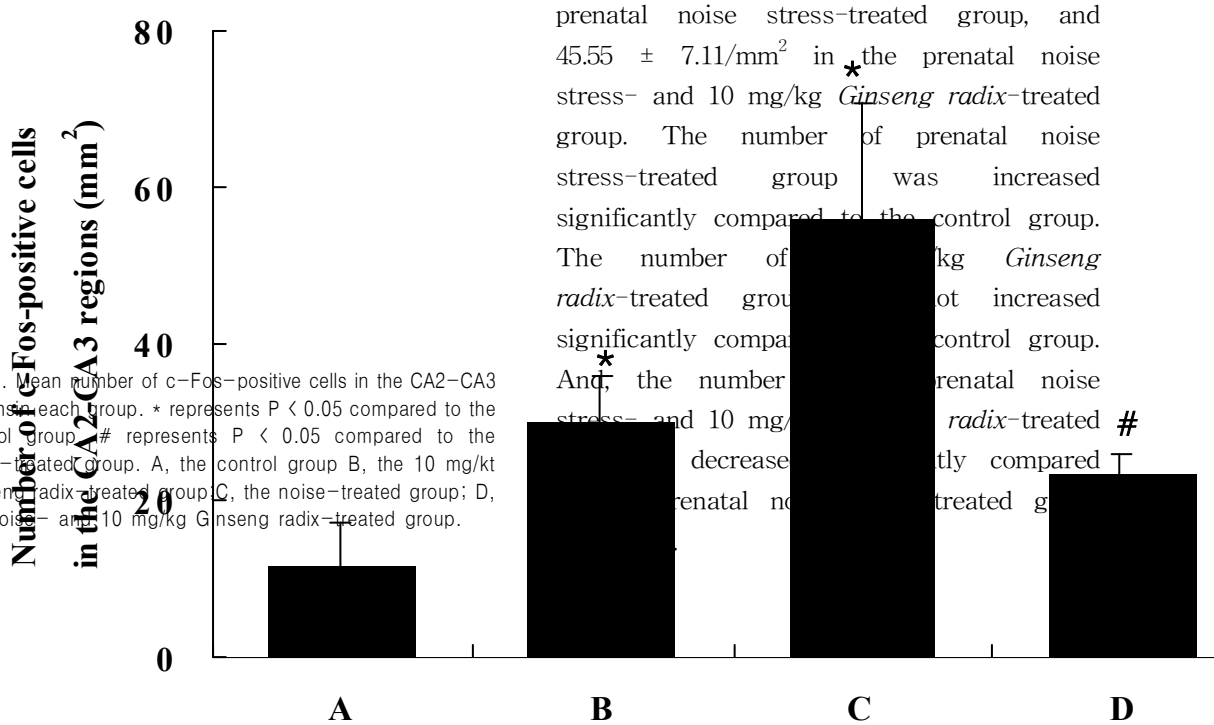


Fig. 3. Mean number of c-Fos-positive cells in the CA2-CA3 regions of each group. * represents P < 0.05 compared to the control group. # represents P < 0.05 compared to the noise-treated group. A, the control group B, the 10 mg/kt Ginseng radix-treated group; C, the noise-treated group; D, the noise- and 10 mg/kg Ginseng radix-treated group.

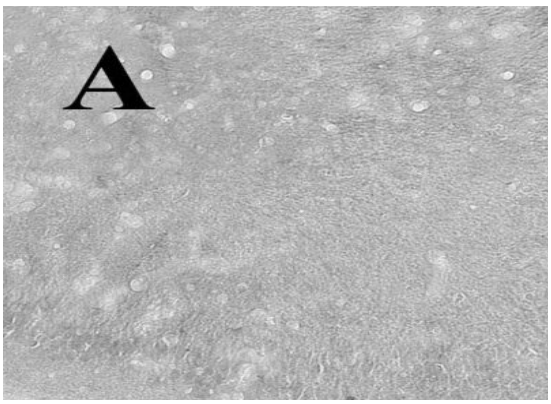


Fig. 4. Photomicrographs of Fos-positive cells in the CA2-3 region in each group. Sections were stained for c-Fos-positive cells (reddish brown). Scale bar represents 100 um. A, the control group B, the 10 mg/kg Ginseng radix-treated group; C, the noise-treated group; D, the noise- and 10 mg/kg Ginseng radix-treated group.

3. Number of c-Fos-positive cells in the dentate gyrus region of hippocampus

The number of c-Fos-positive cells in the dentate gyrus region of hippocampus was 75.39 ± 7.45/mm^2 in the control group, 63.87 ± 12.51/mm^{2}in the 10 mg/kg *Ginseng radix*-treated group, 130.37 ± 9.57 in the prenatal noise stress-treated group, and 45.55 ± 7.11/mm^2 in the prenatal noise stress- and 10 mg/kg *Ginseng radix*-treated group. The number of prenatal noise stress-treated group was increased significantly compared to the control group. The number of kg *Ginseng radix*-treated grou ot increased significantly compa control group. And, the number renatal noise stress- and 10 mg/ *radix*-treated decrease tly compared renatal n treated g

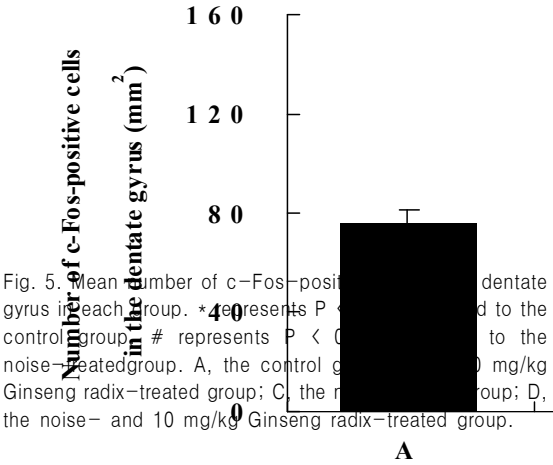


Fig. 5. Mean number of c-Fos-positive cells in the dentate gyrus in each group. * represents P < 0.05 compared to the control group. # represents P < 0.05 compared to the noise-treatedgroup. A, the control group; B, the 10 mg/kg Ginseng radix-treated group; C, the noise-treated group; D, the noise- and 10 mg/kg Ginseng radix-treated group.

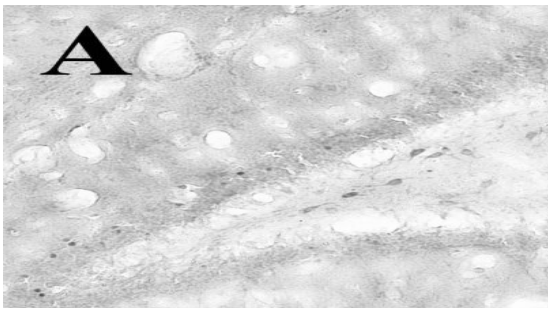


Fig. 6. Photomicrographs of Fos-positive cells in the dentate gyrus region in each group.Sections were stained for c-Fos-positive cells (reddish brown). Scale bar represents 100 um. A, the control group B, the 10 mg/kg Ginseng radix-treated group; C, the noise-treated group; D, the noise- and 10 mg/kg Ginseng radix-treated group.

Ⅳ. DISCUSSION

Stressful experiences during the development period may exert a long-term effect on the hippocampal functions and may induce various psychosomatic problems such as mental retardation and developmental disorders. Various prenatal stresses have been reported to induce structural abnormality in the hippocampal formation. It has been shown that prenatal stress, including both noise stress.[8] and restrained stress[19] during pregnancy, suppress the formation of hipp a variety of mammalian specie on, Coe et al. suggested that p vironment can alter behavior, d neuroendocrine and hippocampal of prima rsistent n uppressio enesis.[8] is induce riety of such as brain injury. In various studies, c-*fos* gene expression has been associated with delayed neuronal cell death, and it has been shown that prolonged c-*fos* induction precedes neuronal death.[20,21] Preston et al. reported that the c-Fos protein plays a causative role in the initiation of apoptosis.[22] In previous study, prenatal stress induces apoptotic neurodegeneration in cerebellum and hippocampus of rats.[23] Saljo et al. suggested that exposure to short-lasting impulse noise causes induction of apoptosis in the hippocampus of adult rat brain.[24] From the present results, it was demonstrated that prenatal noise stress significantly increases the number of Fos-positive cells in the various hippocampal regions.

The aqueous extracts of *Ginseng radix*are composed of a mixture, ginsenosides, trace minerals, and a variety of complex carbohydrates as well as peptides. *Ginsneg radix*was medicinal herb for treatment of various neurodegenerative disorders such as ischemia,[4] Alzheimer's disease,[25] and Parkinson's disease.[26] In addition, administration of *Ginseng radix* was associated with improvement learning and memory. Jin et al. reported that *Ginseng radix* alleviates

scopolamine-induced learning disability and improves spatial working memory in mice.[27] Nishijo et al. suggested that *Ginseng radix* ameliorates learning and memory deficits in an amnesia animal model.[28] In addition, *Ginseng radix* and its constituents are known to possess anti-neoplastic, anti-stress, and anti-oxidant activities.[29-31] Evidence supporting the medicinal efficacy of *Ginseng radix* based on its protective property against free radical attack has been presented.[32] Zhang et al. reported that extracts of *Ginseng radix*scavenge hydroxyl radicals and protect unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation.[33] However, no study on the effect of *Ginseng radix* on the expression of hippocampal neurons containing c-Fosin the offspring rats with prenatal noise stress during pregnancy has been made yet. The present results demonstrated that postnatal *Ginseng radix* administration shown to suppress increments of c-Fos in the CA1, CA2 and CA3, and dentate gyrus of offspring rats with prenatal noise stress during pregnancy. Based on the present study, *Ginseng radix* may provide new therapeutic opportunities as an agent to counteract the effects of prenatal noise stress-induced hippocampal dysfunction through c-Fos change, and may be useful in the treatment of psychiatric problems in children of mothers who have experienced noise stress during pregnancy.

Ⅴ. Conclusion

Exposure to noise during pregnancy adversely influenced the development of the fetus and neonate. In the present study, the influence of postnatal *Ginseng radix* administration on neuronal changes, in particular with respect to c-Fos expression in the hippocampus of offspring rats with prenatal noise stress during pregnancy was investigated *via* c-Fos immunohistochemistry.

From the present results,

1. The number of c - Fos - positive cells in the CA1 region in the prenatal noise stress - and 10 mg / kg Ginseng radix - treated group was decreased significantly compared to the prenatal noise stress - treated group.

2. The number of c-Fos-positive cells in the CA2 and CA3 regions of hippocampus of the prenatal noise stress- and 10mg/kg Ginseng radix-treated group was decreased significantly compared to the prenatal noise stress-treated group.

3. The number of c-Fos-positive cells in the dentate gyrus region of hippocampus of the prenatal noise stress- and 10mg/kg Ginseng radix-treated group was decreased significantly compared to the prenatal noise stress-treated group.

Based on the present study, Ginseng radix may provide new therapeutic opportunities as an agent to counteract the effects of prenatal noise stress-induced hippocampal dysfunction through c-Fos change, and may be useful in the treatment of psychiatric problems in children of mothers who have experienced noise stress during pregnancy.

REFERENCES

1. PaparelliA, Soldani P, Breschi MC, Martinotti E, Scatizzi R, Berrettini S, Pellegrini A. Effects of sub acute exposure to noise on the noradrenergic innervation of the cardiovascular system in young and aged rats: a morphofunctional study. J. Neural. Transm. Gen. Sect. 88, 105-113 (1992).
2. Ravindran R, Devi RS, Samson J, Senthilvelan M. Noise-stress-induced brain neurotransmitter changes and the effect of Ocimum sanctum (Linn) treatment in Albino rats. J. Pharmacol. Sci. 98, 354-360 (2005).
3. Jang MH, Chang HK, Shin MC, Lee TH, Kim YP, Kim EH, Kim CJ. Effect of Ginseng radixon c - Fos expression in the hippocampus of streptozotocin - induced diabetic rats. J. Pharmacol. Sci. 91, 149-152 (2003)
4. Wen TC, Yoshimura H, Matsuda S, Lim JH, Sakanaka M. Ginseng root prevents learning disability and neuronal loss in gerbils with 5-minute forebrain ischemia. Acta Neuropathol. 91, 15-22 (1996)
5. Querleu D, Renard X, Versyp F, Paris-Delrue L, Crepin G. Fetal hearing. Eur. J. Obstet. Gynecol. Reprod. Biol. 28, 191-212 (1988).
6. Sobrian SK, Vaughn VT, Ashe WK, Markovic B, Djuric V, Jankovic BD. Gestational exposure to loud noise alters the development and postnatal responsiveness of humoral and cellular components of the immune system in offspring. Environ. Res. 73, 227-241 (1997).
7. Kay G, Tarcic N, Poltyrev T, Weinstock M. Prenatal stress depresses immune function in rats. Physiol. Behav. 63, 397-402 (1998).
8. Coe CL, Kramer M, Czeh B, Gould E, Reeves AJ, Kirschbaum C, Fuchs E. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile Rhesus monkeys. Biol. Psychiatry 54, 1025-1034 (2003).
9. Eichenbaum H, Otto T, Cohen NJ. The hippocampus: what does it do? Behav. Neural Biol. 57, 2-36 (1992).
10. Jarrard LE. On the role of the hippocampus in learning and memory in the rat. Behav. Neural Biol. 60, 9-26 (1993).
11. Squire LR, Knowlton B, Musen G. The structure and organization of memory. Annu. Rev. Psychol. 44, 453-495 (1993).
12. Dragunow M, Faull R. The use of c-fos as a metabolic marker in neuronal pathway tracing. J. Neurosci. Methods 29, 261-265 (1989).
13. Duncan GE, Knapp DJ, Breese GR. Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. Brain Res. 713, 79-91 (1996).
14. Morgan JI, Curran T. Proto-oncogene transcription factors and epilepsy. Trends Pharmacol. Sci. 12, 343-349 (1991).
15. Goodwin GA, Bliven T, Kuhn C, Francis R, Spear LP. Immediate early gene expression to examine neuronal activity following acute and chronic stressors in rat

pups: Examination of neurophysiological alterations underlying behavioral consequences of prenatal cocaine exposure. Physiol. Behav. 61, 895-902 (1997).
16. Jang MH, Jung SB, Lee MH, Kim H, Lee SJ, Sim YJ, Lee HH, Kim EK, Kim CJ, Shin HS, Kim J, Kim EH. Influence of maternal alcohol administration on c-Fos expression in the hippocampus of infant rats. Neurosci. Lett. 378, 44-48 (2005).
17. Kim EK, Kim H, Lee MH, Kim YS, Yang HY, Chang HK, Lee TH, Jang MH,Shin MC, Lee SJ, Lee HH, Shin MS, Kim CJ. Influence of prenatal noise and music on the 5-hydroxytryptamine synthesis and the tryptophan hydroxylase expression in the raphe nuclei of young rats. Neurosci. Res. Commun. 35, 118-129 (2004).
18. He J, Yamada K, Nabeshima T. A role of Fos expression in the CA3 region of the hippocampus in spatial memory formation in rats. Neuropsychopharmacology 26, 259-268 (2002).
19. Lemaire V, Koehl M, Le Moal M, Abrous DN. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc. Natl. Acad. Sci. USA 97, 11032-11037 (2000).
20. Smeyne RJ, Vendrell M, Hayward M, Baker SJ, Miao G, Schiling K, Roberson LM, Curran T, Morgan JI. Continuous c-Fos expression precedes programmed cell death in vivo. Nature 363, 166-169 (1993).
21. Gubits RM, Burke RE, Casey-McIntosh G, Bandele A, Munell F. Immediate-early gene induction after neuronal hypoxia-ischemia. Mol. Brain Res. 18, 228-238 (1993).
22. Preston GA, Lyon TT, Yin Y, Lang JE, Solomon G, Annab L, Srinivasan DG, Alcorata DA, Barrett JC. Induction of apoptosis by c-Fos protein. Mol. Cell Biol. 16, 211-218 (1996).
23. Ladefoged O, Hougaard KS, Hass U, Sorensen IK, Lund SP, Svendsen GW, Lam HR. Effects of combined prenatal stress and toluene exposure on apoptotic neurodegeneration in cerebellum and hippocampus of rats. Basic Clin. Pharmacol. Toxicol. 94, 169-176 (2004).
24. Saljo A, Bao F, Jingshan S, Hamberger A, Hansson HA, Haglid KG. Exposure to short-lasting impulse noise causes neuronal c-Jun expression and induction of apoptosis in the adult rat brain. J. Neurotrauma 19, 985-991 (2002).
25. Lee TF, Shiao YJ, Chen CF, Wang LC. Effect of ginseng saponins on beta-amyloid-suppressed acetylcholine release from rat hippocampal slices. Planta Med. 67, 634-637 (2001).
26. Kim EH, Jang MH, Shin MC, Shin MS, Kim CJ. Protective effect of aqueous extract of Ginseng radixagainst 1-methyl-4-phenylpyridinium-induced apoptosis in PC12 cells. Biol. Pharm. Bull. 26, 1668-1673 (2003).
27. Jin SH, Park JK, Nam KY, Park SN, Jung NP. Korean red ginseng saponins with low ratios of protopanaxadiol and protopanaxatriol saponin improve scopolamine-induced learning disability and spatial working memory in mice. J.Ethnopharmacol. 66,123-129 (1999).

28. Nishijo H, Uwano T, Zhong YM, Ono T. Proof of the mysterious efficacy of ginseng: basic and clinical trials: effects of red ginseng on learning and memory deficits in an animal model of amnesia. J. Pharmacol.Sci. 95, 145-152 (2004).
29. Attele AS, Wu JA, YuanCS. Ginseng pharmacology: multiple constituents and multiple actions. Biochem. Pharmacol. 58, 1685-1693 (1999).
30. Grillis CN. Panax ginseng pharmacology: a nitric oxide link?. Biochem. Pharmacol. 54, 1-8 (1997).
31. Kim YK, Guo Q, Packer L. Free radical scavenging activity of red ginseng aqueous extracts. Toxicology 172, 149-156 (2002).
32. Maffei Facino R, Carini M, Aldini G, Berti F, Rossoni G. Panax ginseng administration in the rat prevents myocardial ischemia-reperfusion damage induced by hyperbaric oxygen: evidence for an antioxidant intervention. Planta Med. 65, 614-619 (1999).
33. Zhang D, Yasuda T, Yu Y, Zheng P, Kawabata T, Ma Y, Okada S. Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. Free Radic. Biol. Med. 20, 145-150 (1996).