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Influence of prenatal noise and music on the spatial memory and neurogenesis in the hippocampus of developing rats

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Abstract

During the prenatal period, the development of individual is influenced by the environmental factors. In the present study, the influence of prenatal noise and music on the spatial memory and neurogenesis in the hippocampus of developing rats was investigated. The exposure to the noise during pregnancy caused growth retardation, decreased neurogenesis in the hippocampus, and impaired spatial learning ability in pups. The exposure to music during pregnancy, on the other hand, caused increased neurogenesis in the hippocampus and enhanced spatial learning ability in pups. The present study has shown the importance of the prenatal environmental conditions for the cognition and brain development.
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1. Introduction

During the prenatal period, the development of individual is influenced by the environmental factors. Various physical and emotional stresses applied to mothers result in low birth-weight of the offspring, increased risk of premature delivery, and the higher incidence of neonatal abnormality [1]. In addition, delayed motor and cognition development in the offspring of stressed pregnant rats has been reported [2].

In the late gestation, the fetus can hear the sound from the outside of mother [3]. Previous studies reported that the exposure to noises during pregnancy adversely influenced the development of the fetus and neonate. Increased antepartum fetal death, congenital anomaly in the central nervous system, impaired social behavior in juvenile stage,

and the long-term alteration in the immune function have been reported [4,5].

In contrast, the prenatal exposure to music has been suggested to be beneficial to the fetal development. Music stimulation during pregnancy was reported to enhance the brain development of the fetus, improve spatial-temporal learning in neonatal rats, and induce the rapid advance in motor ability such as sitting and walking in baby [6–8].

The ability to remember and use of the information on the distribution of food and other resources is vital for animal's survival [9]. The recalling ability of the location in the physical environment is termed spatial memory. The hippocampal formation, that begins in the gestation stage and continues to the postnatal period [10], is critical for animals to use spatial information to guide and organize their behavior [11]. It has been demonstrated that the loss of cells in the hippocampus impairs the spatial learning and spatial memory capability [12]. In contrast, the generation of new neurons in the hippocampus has been shown to be associated with certain types of memory formation [13].

It is well known that the exposure to various stimuli affects neurogenesis in the hippocampus of developing and adult mammals [14]. In the present study, the influence of

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prenatal noise and music on the spatial memory and neurogenesis in the hippocampus of developing rats was investigated.

2. Materials and methods

2.1. 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 °C) and the light–dark cycle of 12 h of light and 12 h of darkness (light on from 07:00 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 three groups: the control group, the noise-applied group, and the music-applied group ($n = 5$ in each group).

From the 15th day of pregnancy until the delivery, all rats were subcutaneously injected with 100 mg/kg 5-bromo-2′-deoxyuridine (BrdU: Sigma Chemical Co., St. Louis, MO) once a day 30 min before the starting of experimental treatment until delivery. The noise-applied rats were exposed to the 95 dB supersonic machine sound for 1 h once a day until delivery. The music-applied rats were exposed to the 65 dB comfortable music sound for 1 h once a day until delivery. Control rats were left undisturbed. After birth, the offspring were left undisturbed together with their mother. The spatial learning test was performed on the 21 days after birth. Animals were sacrificed immediately after the completion of the spatial learning test.

2.2. Radial-arm maze test

The spatial learning ability was tested using a radial-arm maze apparatus as previously described [15]. The radial-arm maze apparatus consisted of a central octagonal plate (30 cm in diameter) and radiating eight arms (50 cm in length and 10 cm in width). The apparatus was placed 1 m above the floor. A small receptacle filled with water (3 cm in diameter and 1 cm in depth) was located at the end of the arms. Rats were trained three times before the spatial learning test. Rats deprived of water for 24 h were allowed to explore for water and to drink for 5 min. On the 21 days after birth, the spatial learning ability test was performed. The time spent for the seeking of water at the end of the arms was counted. The test was terminated when a rat found water in all eight arms or over 5 min elapsed. Re-entering to the previously visited arm was counted as error. In addition,

the number of correct choice before the first error was counted.

2.3. Tissue preparation

For brain tissue preparation, animals were fully anesthetized with Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France), transcardially perfused with 50 mM phosphate-buffered saline (PBS), and fixed with the freshly prepared solution consisting of 4% paraformaldehyde (PFA) in 100 mM phosphate buffer (PB, pH 7.4). The brains were then removed, post-fixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Coronal sections of 40 μm thickness were made using a freezing microtome (Leica, Nussloch, Germany).

2.4. BrdU immunohistochemistry

For the detection of newly generated cells in the dentate gyrus, BrdU incorporation that has been generally used as the indicator of DNA synthesis was visualized using the immunohistochemical method as previously described [16]. Briefly, sections were permeabilized by incubating with 0.5% Triton X-100 in PBS for 20 min, treated with 50% formamide-$2 \times$ standard saline citrate (SSC) at 65 °C for 2 h, denatured in 2 N HCl at 37 °C for 30 min, and rinsed twice in 100 mM sodium borate (pH 8.5). Subsequently, the sections were incubated overnight at 4 °C with BrdU-specific mouse monoclonal antibody (1:600; Roche, Mannheim, Germany). The sections were then washed three times with PBS and incubated for 1 h with a biotinylated mouse secondary antibody (1:200; Vector Laboratories, Burlingame, CA). Then, the sections were incubated for another 1 h with avidin–peroxidase complex (1:100; Vector Laboratories). For the visualization of BrdU, the sections were incubated with 50 mM Tris–HCl (pH 7.6) containing 0.02% 3,3′-diaminobenzidine containing nickel chloride (40 mg/ml) and 0.03% hydrogen peroxide for 5 min.

After BrdU-specific staining, counter-staining was performed on the same sections using a mouse anti-neuronal nuclei (NeuN) antibody (1:300; Chemicon International, Temecula, CA, USA). The sections were washed three times with PBS, incubated for 1 h with a biotinylated anti-mouse secondary antibody, and processed with VECTASTAIN® ABC Kit. For staining, the sections were reacted with 0.02% DAB and 0.03% hydrogen peroxide in 50 mM Tris–HCl (pH 7.6) for 5 min and the sections were finally mounted onto gelatin-coated slides. The slides were air dried overnight at room temperature, and coverslips were mounted using Permount®.

2.5. Data analysis

The area in the selected region of the hippocampus was measured using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The number of BruU-positive

cells in the areas in the hippocampus was counted hemilaterally. The data were expressed as the number of cells per square millimeter in the area of the hippocampus. Statistical analysis was performed using one-way ANOVA followed by Duncan *post-hoc* test. The results are presented as the mean $\pm$ SEM. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Body weight

The mean body weight at 2 weeks after birth of the control group was 27.80 ± 0.75 g, the noise-applied group was 19.42 ± 0.50 g, and the music-applied group was 27.07 ± 0.97 g. The significant retardation of body weight gain was observed in the noise-applied group. In contrast, significant difference in the body weight of the music-applied group and the control group was not detected.

3.2. Influence of prenatal noise and music on spatial memory of rat offspring

The control rats completed eight successful performances in 110.88 ± 14.42 s, the noise-applied group completed in 122.80 ± 14.74 s, and the music-applied group completed in 63.00 ± 7.73 s. The number of correct choice before the first error in the control group was 6.44 ± 0.29, the noise-applied group was 5.40 ± 0.47, and the music-applied group was 6.90 ± 0.23. The number of error made before eight successful performances in the control group was 5.55 ± 1.00, the noise-applied group was 13.20 ± 3.29, and the music-applied group was 3.20 ± 0.85 (Fig. 1). Our data demonstrate that in comparison with the control rats, the noise-applied rats showed the lower number of correct choice and had the higher number of error. In contrast, the music-applied rats spend less time for the seeking of water in eight arms.

3.3. Influence of prenatal noise and music on the hippocampal neurogenesis of rat offspring

Fig. 2 shows BrdU-positive cells in various regions of the hippocampus. The number of BrdU-positive cells in the hippocampal CA1 region of the control group was $2352.00 \pm 111.40/mm^2$, the noise-applied group was $1644.22 \pm 93.70/mm^2$, and the music-applied group was $3229.59 \pm 119.04/mm^2$. The number of BrdU-positive cells in the hippocampal CA2 and CA3 regions of the control group was $868.00 \pm 40.50/mm^2$, the noise-applied group was $748.85 \pm 59.12/mm^2$, and the music-applied group was $1393.70 \pm 57.66/mm^2$. The number of BrdU-positive cells in the hippocampal dentate gyrus of the control group was $2367.28 \pm 138.25/mm^2$, the noise-applied group was
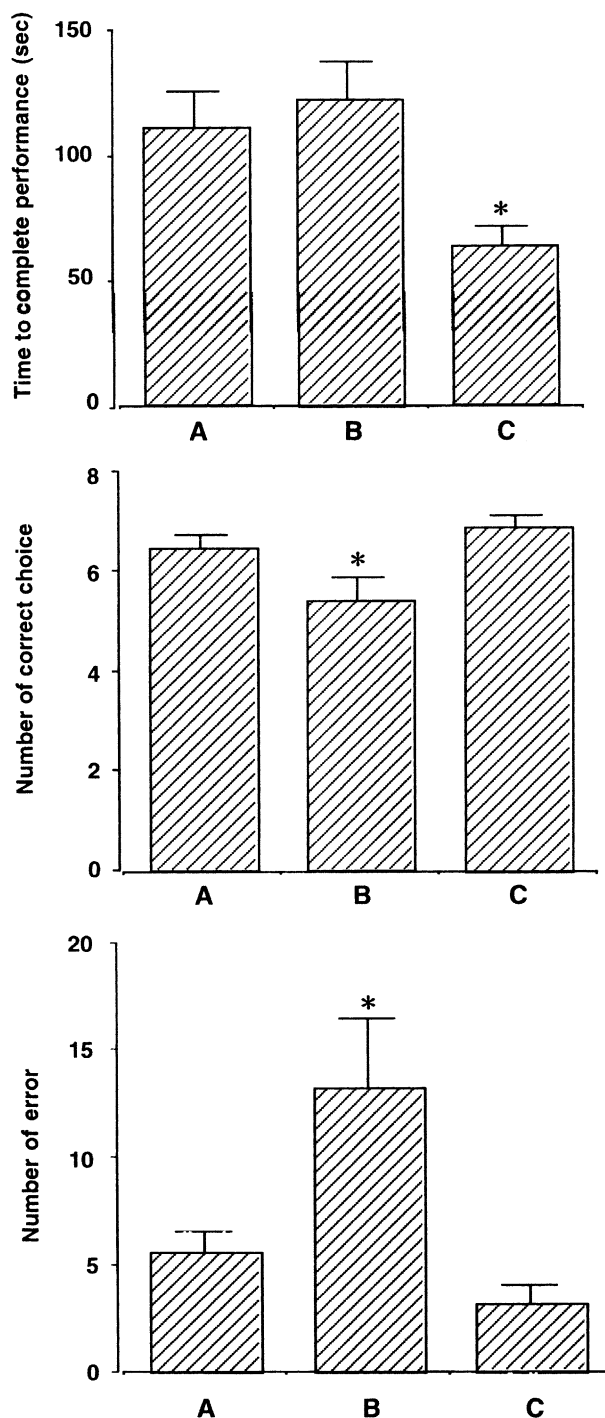

Fig. 1. The influence of prenatal noise and music on the spatial memory. Upper: the time to complete eight successful performances. Middle: the number of correct choice prior to the occurrence of first error. Lower: the number of error prior to eight successful performances. The data are presented as the mean $\pm$ SEM. *$P < 0.05$ compared to the control group. (A) Control group, (B) noise-applied group, (C) music-applied group.

$1170.91 \pm 146.76/mm^2$, and the music-applied group was $2055.72 \pm 124.39/mm^2$ (Fig. 3).

Our data demonstrate that in comparison with the control rats, the noise-applied group showed significantly reduced

Fig. 2. Photomicrographs of 5-bromo-2′-deoxyuridine (BrdU)-positive cells in the hippocampus. Sections were stained for BrdU (black) and neuronal nuclei (NeuN; brown). The scale bar represents 500 μm. (A) Control group, (B) noise-applied group, (C) music-applied group (For interpretation of the reference to color in this legend, the reader is referred to the web version of this article).

neurogenesis in the CA1 region and the dentate gyrus. In contrast, the music-applied group showed enhanced neurogenesis in the CA1 region and the CA2 and CA3 regions.

4. Discussion

Here, we investigated the influence of prenatal noise and music on the spatial learning ability and hippocampal neurogenesis of the offspring. It has been suggested that 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 [10,17].

Pups of rats exposed to cold water immersion or received psychosomatic stress induced by a cat showed growth retardation [2,18]. Reduced uterine blood flow causing fetal hypoxemia was suggested as the mechanism of growth retardation of pups [2]. We also observed the significant growth retardation in the pups of rats received stressful sound during the late pregnancy period.

Various prenatal stresses have been reported to induce structural abnormality in the hippocampal formation. It was reported that prenatal stress reduced the density of the pyramidal neurons and nitric oxide-producing neurons, decreased the total hippocampal volume, and induced the synaptic loss in the hippocampus [1,19]. Decreased neurogenesis in the dentate gyrus was reported in the pups of rats received restraint-induced stress during pregnancy [11]. In the present study, hippocampal neurogenesis was decreased in the pups of rats exposed to stressful noise during the late gestational stage.

Prenatal stresses are known to alter the responses of the hypothalamo-pituitary-adrenal (HPA) axis [20] and induce cognitive deficit or impairment of offspring [21]. Hayashi et al. showed that maternal stress induced by crowding and saline injection decreased the ability of the pups to cope with new situations [1]. The spatial learning ability in the water maze test was decreased in male rats received prenatal stress [17]. The present results showed that the exposure to the noise during pregnancy caused the decrease of the spatial learning ability of pups.

Although the precise mechanism leading from prenatal stress to cognitive deficit in the offspring remains poorly understood, a few possible presumptions could be considered. In this study, we performed spatial learning task with developing rat of postnatal day 21. Neural circuits of immature brain in developing rats of postnatal day 21–22 are still excitatory dominant due to the late development of inhibitory circuits. Brain of developing rats used to perform the behavioral test is rapidly shifting from an immature function to a mature one following the weaning period. Therefore, it is quite likely that the abnormalities in learning ability observed in the pups of postnatal day 21 are resulted from the delayed development of cognitive function in pups with prenatal noise-enrichment or its facilitated development in pups with prenatal music-enrichment.

On the other hand, the present result showed that the effect of prenatal stress on cognitive deficit may be due to the decrease of neurogenesis in the hippocampus induced by noise. It is well known that the hippocampal formation plays a pivotal role in learning ability and memory capability. Furthermore, the generation of new neurons in the hippocampus has been shown to be essential for the maintaining of normal learning and memory process [13,22]. Increased neurogenesis in the dentate gyrus of rats improved their learning ability [13], while stress and aging that reduce cell proliferation impaired learning and memory functions [23].

Previous studies have shown that prenatal stress increases maternal corticosterone which readily penetrates to the fetal brain [24]. Stress-induced corticosterone is known to decrease hippocampal neurogenesis [14] and impairs learning ability and memory capability [25]. The animals received prenatal stress showed a delayed habituation of the corticosterone response to repeated exposure to stress [26]. Recently, Lemaire et al. reported that maternal stress induces smaller hippocampus and spatial learning deficit due to the impairment of offspring's neurogenesis [17]. In the present results, developing rats received noise stress during prenatal period showed worse memory scores in the spatial learning task and decreased neurogenesis in the hippocampus. In this study, we have shown that hippocampal neurogenesis is closely related to the spatial learning ability.
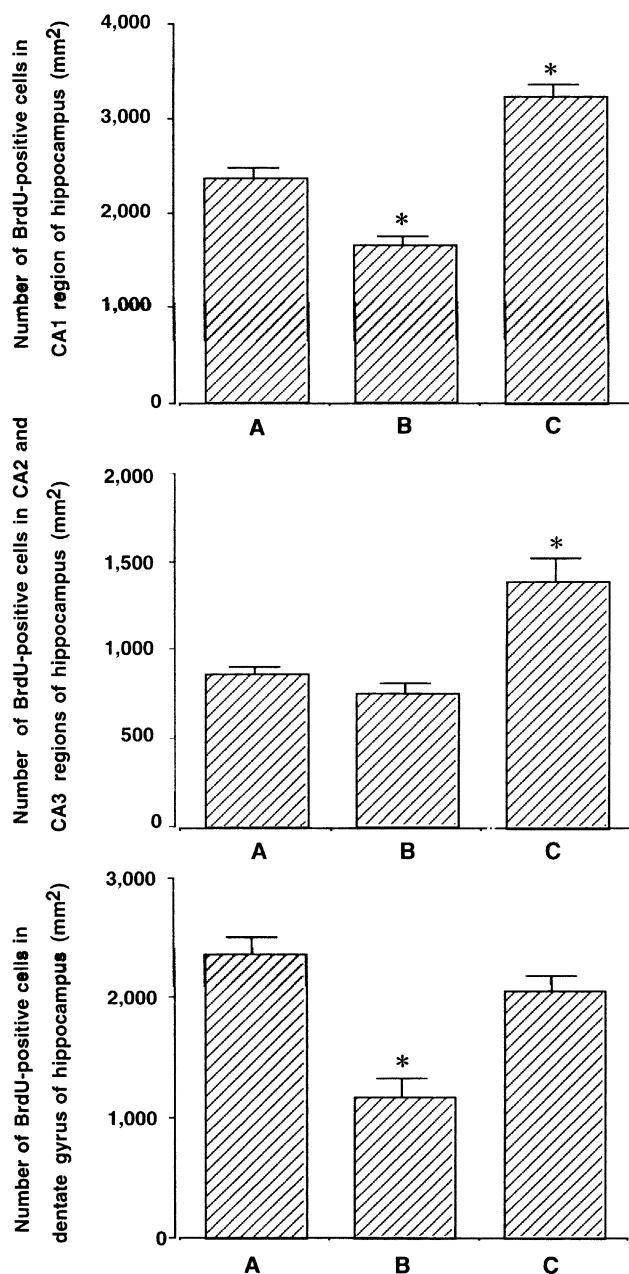
Fig. 3. The influence of prenatal noise and music on the neurogenesis in the hippocampus. The data are presented as the mean ± SEM. *$P < 0.05$ compared to the control group. Upper: the number of 5-bromo-2′-deoxyuridine (BrdU)-positive cells in the CA1 region of the hippocampus. Middle: the number of BrdU-positive cells in the CA2 and CA3 regions of the hippocampus. Lower: the number of BrdU-positive cells in the dentate gyrus of the hippocampus.

It is generally accepted that music is very effective for prenatal education. Numerous studies have suggested that the exposure to music during pregnancy may facilitate fetal growth and brain development [27]. The prenatal music sound stimulation enhanced the synaptic protein expression in the brainstem auditory nuclei and increased the size and the number of neurons in the forebrain auditory association area of the chicks [8,28]. Newborn babies exposed to

musical stimulus during pregnancy showed significant improvement in autonomic stability and were more easily comforted [29]. Infants who had been exposed to music stimulation during pregnancy showed the rapid development of motor ability such as sitting and walking [6]. Rauscher et al. reported that rats exposed to Mozart's music during pregnancy and 60 days post-partum completed the T-maze task more rapidly with fewer errors, which shows improved the spatial-temporal learning ability [7]. In the present results, pups exposed to music during prenatal period showed the increase of neurogenesis in the hippocampus, resulting in enhancing spatial learning ability.

Here, we have demonstrated that prenatal noise caused growth retardation, decreased neurogenesis in the hippocampus, and impaired spatial learning ability in pups. In contrast, prenatal music caused the increase of neurogenesis in the hippocampus and enhanced spatial learning ability in pups. The present study has shown the importance of the prenatal environmental conditions for the cognition and brain development.

Acknowledgements

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