Influence of Prenatal Noise and Music on the Expressions of c-Fos and Nitric Oxide Synthase in the Hippocampus of Rat Pups

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The expressions of c-Fos and nitric oxide synthase (NOS) represent neuronal activity and play a crucial role in the shaping of the development of brain. During the late pregnancy, stresses may influence neuronal activity of prenatal rats. In the present study, the effects of prenatal noise and music on the expressions of c-Fos and NOS in the hippocampus of rat pups were investigated. Exposure to the noise during pregnancy decreased c-Fos and NOS expressions in the hippocampus of rat pups, whereas exposure to music during pregnancy increased c-Fos and NOS expressions in the hippocampus of rat pups. The present results show that prenatal music stimulation may increase neuronal activity of rat offspring, whereas exposure to noise during pregnancy may reduce the neuronal activity of offspring. The present study suggests that prenatal stimuli including noise and music could affect the fetal brain development.

Key words : Prenatal noise, prenatal music, c-Fos, nitric oxide synthase, hippocampus

Introduction

It is well known that many factors such as viral infection, drugs and alcohol abuse, endocrine impairment, food deprivation, and stress during pregnancy may induce physical malformation, behavioral dysfunction, and developmental retardation of offspring[1-5]. Of these, prenatal stresses have been suggested to induce growth retardation, impairment of memory capability, and behavioral disorders of rat pups[1,4,6]. In contrast, prenatal exposure to music sound has been suggested to enhance synapse formation of auditory nuclei in chickens and to improve spatial-temporal learning ability of neonatal rat pups[7,8]. Arabin (2002) suggested that musical stimulation during pregnancy favors fetal development and prevents sensory retardation in neonate and infant[9].

Hippocampal formation is the brain region critically involved in learning and memory formation. The expression of specific immediate-early genes (IEGs) is essential for the neuroplasticity requiring memory consolidation process[10]. Of these, c-Fos expression is essential for encoding spatial memory in the hippocampus and a marker for neuronal activity in early transcriptional event in the stress-responsive areas of the brain[11,12]. In addition, hippocampus-mediated learning enhances generation of new neurons in the dentate gyrus[13].

Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) which converts L-arginine to L-citrulline. Availability of arginine is one of the rate-limiting factors for the cellular NO production[14]. Several isoforms of NOS exist and fall into three major classes: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). Of these, nNOS is mainly expressed in the central nervous system (CNS) and has also been implicated in signal transmission[15,16].

Spatial learning and memory induce up-regulation of c-Fos and nitric oxide synthase (NOS) expressions in rat brains[11,17,18]. These studies suggested that expressions of c-Fos and NOS represents neuronal activity and plays a crucial role in the shaping of the developing brain. However, the effect of noise stress and music sound during pregnancy on the brain development of the fetus is a controversial issue. In particular,

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little is known about the effect of noise and music during pregnancy on the expressions of c-Fos and NOS in the hippocampus of offspring.

In the present study, the influence of noise and music during pregnancy on the expressions of c-Fos and NOS in the hippocampus of rat pups was investigated using c-Fos immunohistochemistry and the nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) histochemistry which provides a specific histochemical marker for NOS, respectively[15].

Materials and Methods

1. Animals and treatments

Female Sprague-Dawley rats (180 ± 10 g, 8 weeks old, n = 15) were used in the present study. The experimental procedures were performed in accordance with the guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. Female rats pre-delivery were housed individually in a plastic home cage for 2 weeks at a controlled temperature (20 ± 2℃) and maintained under light-dark cycles consisting of 12 h of light and 12 h of darkness (lights on from 07:00 h to 19:00 h). Food and water were made available ad libitum. After confirming of pregnancy, 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). The noise-applied rats were exposed to the 95-desibel (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 neonatal rats were left with the respective mothers. The rats were sacrificed on the 28 days after birth.

2. Tissue preparation

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

3. c-Fos immunohistochemistry

c-Fos immunohistochemistry was perfused as previously described[19]. Eight sections on average within the hippocampal region spanning from Bregma -3.30 to -4.16 mm were obtained from each brain. Free-floating tissue sections were incubated overnight with rabbit anti-c-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1 : 1000, for visualization of c-Fos distribution. The sections were then incubated for 1 h with anti-rabbit secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA) for anti-c-Fos immunohistochemistry. The sections were subsequently incubated with avidin-biotin-peroxidase complex (1:100, Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution containing 0.05% 3,3-diaminobenzidine (DAB) and 0.01% H_2O_2 in 50mM Tris-buffer (pH 7.6) for approximately 3min.

As the negative control, the brain sections were likewise processed using normal goat serum as the primary antibody. No c-Fos-like immunoreactivity was observed.

4. NADPH-d histochemistry

NADPH-d histochemistry was performed as previously described[20]. In brief, free-floating sections were incubated at 37℃ for 60 min in 100 mM PB (pH 7.4) containing 0.3% Triton X-100, 0.1 mg/ml nitroblue tetrazolium, and 0.1 mg/ml β-NADPH. The sections were then washed three times with PBS and mounted onto gelatine-coated slides. The slides were air dried overnight at room temperature, and coverslips were mounted using Permount®.

5. Data analysis

The area of the dentate gyrus of hippocampus was measured using an image analyzer (Multiscan, Fullerton, CA, USA). The numbers of NADPH-d-positive cells and c-Fos positive cells were counted hemilaterally. The data were expressed as the number of cells per mm^2 of cross-sectional area of the selected region.

6. Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Scheffé's post-hoc test. The results were expressed as the mean ± standard error of the mean (S.E.M.). Difference was considered significant at $p<0.05$.

Results

1. Body weight gain

The body weight of offspring is presented in Fig. 1. Body weight of offspring at 28 days after birth was 115.00 ± 0.95 g in the control group, the noise-applied group was 91.50 ± 0.60 g, and the music-applied group was 113.80 ± 0.87 g(Fig. 1).

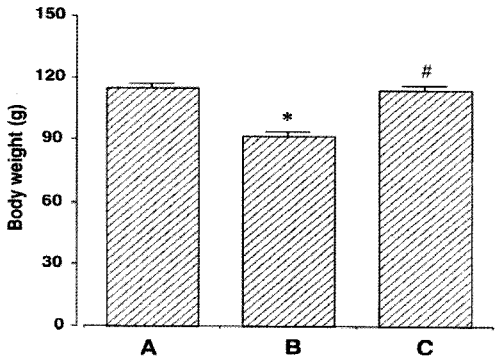

Fig. 1. Body weight of offspring at 28 days after birth. (A) Control group, (B) noise-applied group, (C) music-applied group. Values are represented as the mean ± S.E.M. * represents p<0.05 compared to the control group. # represents p<0.05 compared to the noise-applied group.

2. c-Fos expression in the hippocampus

Fig. 2 shows c-Fos-positive cells in the hippocampus. The number of c-Fos-positive cells in the hippocampal CA was decreased in the noise-applied group and it was increased in the music-applied group. The number of c-Fos-positive cells in the hippocampal dentate gyrus was decreased in the noise-applied group and it was increased in the music-applied group(Fig. 2). Noise stress during late pregnancy suppressed c-Fos expression in the hippocampus of offspring. Music stimulus, however, enhanced c-Fos expression in the hippocampus of offspring.

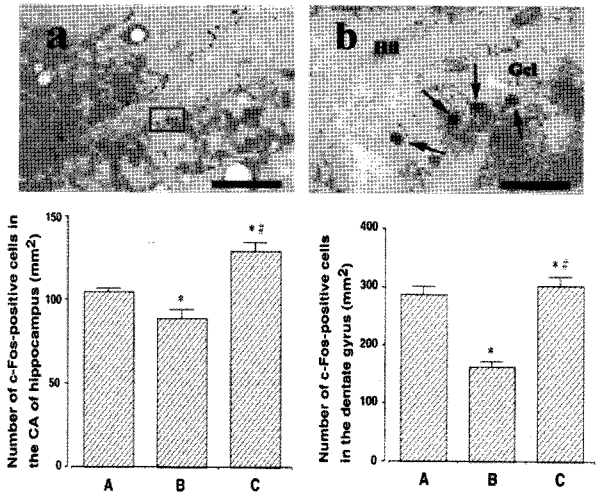

Fig. 2. Influence of prenatal noise and music stimulation on c-Fos expression in the hippocampus. Upper: Photomicrographs of c-Fos expression in the dentate gyrus of hippocampus. The scale bar represents 100 μm (a) and 25 μm (b). Black arrows indicate c-Fos-positive cells. Gcl, Granular cell layer; Hil, Hilus. Middle: The mean number of c-Fos-positive cells in CA of hippocampus. (A) Control group, (B) noise-applied group, (C) music-applied group. Lower: The mean number of c-Fos-positive cells in the dentate gyrus of hippocampus. (A) Control group, (B) noise-applied group, (C) music-applied group. Values are represented as the mean ± S.E.M. * represents p<0.05 compared to the control group. # represents p<0.05 compared to the noise-applied group.

3. NOS expression in the hippocampus

NADPH-d-positive cells in the hippocampus are presented in Fig. 3. The number of NADPH-d-positive cells in the hippocampal CA was decreased in the noise-applied group and it was increased in the music-applied group. The number of NADPH-d-positive cells in the hippocampal dentate gyrus was decreased in the noise-applied group and it was increased in the music-applied group(Fig. 3). Noise stress during late pregnancy suppressed NOS expression in the hippocampus of offspring. Music stimulus, however, enhanced NOS expression hippocampus of offspring.

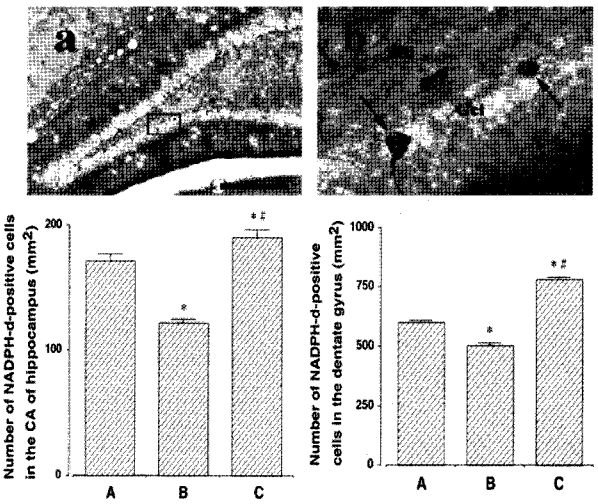

Fig. 3. Influence of prenatal noise and music stimulation on nitric oxide synthase (NOS) expression in the hippocampus. Upper: Photomicrographs of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-positive cells in the dentate gyrus of hippocampus. The scale bar represents 100 μm (a) and 25 μm (b). Black arrows indicate NADPH-d-positive cells. Gcl, Granular cell layer; Hil, Hilus. Middle: The mean number of NADPH-d-positive cells in the CA of hippocampus. (A) Control group, (B) noise-applied group, (C) music-applied group. Lower: The mean number of NADPH-d-positive cells in the dentate gyrus of hippocampus. (A) Control group, (B) noise-applied group, (C) music-applied group. Values are represented as mean ± S.E.M. * represents p<0.05 compared to the control group. # represents p<0.05 compared to the noise-applied group.

Discussion

In the present study, prenatal noise-induced stress retarded growth rate after birth. Significant retardation of body weight gain was observed in the noise-treated group, in contrast no significant difference was observed in body weight between the music-applied group and the control group.

Retana-Marquez et al. (2003) also reported that body weight gain was decreased in stressed rats compared to normal rats[21]. Many physical stimuli are known to induce alternation of brain structures including atrophy of hippocampus. Severe stress during pregnancy may disturb normal development of postnatal rats, resulting in delay of emotional behavior and learning ability, temperament character, and altering of fetal brain function permanently[2,3,5,22]. Exposure to stresses such as noise, immobilization, and the presence of a cat induced brain damage, retardation of auditory cortical development, and impairment of growth

rate[1,23-25]. In addition, stress inhibited neuronal activity in the brain and suppressed neurogenesis[26-28].

Stress-induced activation of the hypothalamic-pituitary-adrenal axis has been reported to interfere with hippocampal cell proliferation, giving rise to anatomical abnormalities and pathological response to stress[29,30]. Veenema et al. (2004) reported that the regulation of the hypothalamus-pituitary-adrenal (HPA) axis and the serotonin (5-HT) system was increased in long attack latency mice under baseline and stress conditions and the hippocampal cell proliferation rate was decreased in long attack latency mice. Exposure to acute stress resulted in a hyper-reactive HPA regulation, an increased 5-HT neurotransmission, and a decrease of hippocampal cell proliferation rate[31].

It is generally accepted that hearing music during pregnancy exerts a profound and long-term effect on the child. Music sound also alleviates stress-induced suppression on immune function in mice and suppresses stress-induced enhancing of lung cancer metastasis[32]. In addition, music has been considered to exert emotional benefit and it was reported to increase spatial-temporal reasoning[33].

In the present study, c-Fos expression in the hippocampus was decreased in the pups born from the rats receiving noise during late pregnancy. c-Fos expression, on the other hand, was increased in the pups born from the rats receiving music during late pregnancy.

Immediately early genes (IEGs) including c-Fos in the hippocampus were rapidly expressed in response to several stimuli. The expression of IEGs is considered to play a role in the neuroplastic mechanism[10]. Suppression of c-Fos expression was shown to impair long-term memory consolidation[34]. Enhancing of c-Fos expression is known to increase neuronal activity, which leads to increasing spatial learning ability and memory capability[35].

In the present study, NOS expression in the hippocampus was decreased in the pups born from the rats receiving noise during late pregnancy. NOS expression, on the other hand, was increased in the pups born from the rats receiving music during late pregnancy.

NO may serve as an inter-neuronal messenger that modulates neurotransmitter release in mammalian brain and activates NOS enzyme. Sanchez et al. (1999) showed a significant increase in the NOS expression following exposure to stress, such as forced swimming, especially after 30 min[36]. The expression of NOS represents a marker of neuronal activity[16]. Holsche et al (1996) showed that selective neuronal NOS inhibitors impair spatial learning and memory formation in rats[37]. In addition, it has been suggested that NO is associated with neurogenesis in the adult mice[38].

Acute mild stressful experiences facilitate consolidation of new memories and promote cognitive process. In contrast, adverse experiences, particularly when severe and persistent, may induce neuronal dysfunctions and neuronal loss. Here in this study, we have shown that the prenatal music exposure enhanced c-Fos and NOS expressions in the hippocampus of young rats, while prenatal noise exposure suppressed c-Fos and NOS expressions in the hippocampus of young rats. The present results suggest that prenatal stimuli including noise and music may affect fetal brain development.

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