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● *Review*

# PRENATAL EXPOSURE TO ULTRASOUND AFFECTS LEARNING AND MEMORY IN YOUNG RATS

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**Abstract—Prenatal exposure to ultrasound may cause cognitive impairments in experimental animals; however, the exact mechanisms remain unknown. In this study, we exposed pregnant rats (or sham-exposed controls) to different intensities of ultrasound repeatedly on days 6, 12 and 18 of pregnancy for 4 min (3.5 MHz, spatial peak time average intensity = 7.6 mW/cm$^2$, mechanical index = 0.1, thermal index bone = 0.1: 4-min group) or 20 min (3.5 MHz, spatial peak time average intensity = 106 mW/cm$^2$, mechanical index = 1.4, thermal index bone = 1.0: 20-min group). The Morris water maze was used to assess learning and memory function in pups at 2 mo of age. Noticeable deficits in behavior occurred in the group exposed to ultrasound for 20 min. Using real-time polymerase chain reaction and Western blot, we also determined that both the mRNA and protein expression levels of hippocampal *N*-methyl-D-aspartate (NMDA) receptor units 1 (NR1) and 2B (NR2B) and brain-derived neurotrophic factor (BDNF) were significantly lower in pups exposed to ultrasound for 20 min than in controls. Furthermore, the morphology of the synapses in the hippocampus was partially damaged. Compared with the control group, the 4-min group had better spatial learning and memory abilities, as well as higher mRNA and protein levels of NR1, NR2B and BDNF. Our study suggests that high-intensity ultrasound irradiation can decrease learning and memory abilities by reducing the expression of NR1, NR2B and BDNF in the hippocampal regions and damaging the structure of synapses. In contrast, low-intensity ultrasound irradiation can enhance the learning and memory abilities of the offspring rats by increasing the expression of NR1, NR2B and BDNF receptor in the hippocampal regions. (E-mail: wangpeijun_918@126.com)** **© 2015 World Federation for Ultrasound in Medicine & Biology.**

***Key Words:*** **Ultrasound, Hippocampus, Learning and memory, Morris water maze, *N*-methyl-D-aspartate, Brain-derived neurotrophic factor, Receptor, Synapses.**

## INTRODUCTION

Ultrasound examination is an irreplaceable prenatal diagnostic method. There is a tendency toward multiple, early applications of ultrasound in obstetrics because of its non-invasiveness, simplicity, dynamics and reproducibility (Ijaiya et a1. 2002). Given that developing nervous system tissues are sensitive to ultrasound exposure (Barnett et al. 1997) and the output power of modern ultrasound machines has increased exponentially in the past decade, it is important to assess the tolerability of ultrasound use and the potential for long-term effects, such as decreased cognitive function.

Ultrasound can affect neuronal migration in developing fetal mouse brains (Ang et al. 2006). In rats, ultrasound exposure during the crucial period of embryonic development may impair brain function and alter the release of transmitters. Devi et al. (1995) reported that exposure of 14.5-d pregnant Swiss Albino rats to diagnostic ultrasound for 10 min affected the movement and learning abilities of the offspring rats. Suresh et al. (1996, 2002) used diagnostic ultrasound to irradiate 14-d pregnant rats for 10, 20 and 30 min and tested the behavior of their offspring 4 and 12 mo after birth. At 4 mo after birth, previous exposure to ultrasound longer than 10 min had impaired learning abilities in the offspring; these changes in learning and memory abilities were no longer evident 12 mo after birth. Suneetha and Kumar (1993) used low-frequency ultrasound to irradiate pregnant rats for 5–7 min and found that their acetylcholinesterase levels increased. Suresh et al. (2008) used diagnostic ultrasound to irradiate 14- and 16-d pregnant rats and found that the learning and memory abilities of their offspring decreased. Further, coronal sections of

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the dorsal hippocampus revealed a decrease in the number of neurons in the CA3 and CA4 regions; levels of noradrenaline, dopamine, serotonin and the serotonin metabolite 5-hydroxyindoleacetic acid in the hippocampus also decreased.

The hippocampus plays an important role in learning and memory (Squire et al. 2004). Hippocampal damage results in various behavioral alterations (Baskar and Devi 2000; Hossain and Uma Devi 2001; Sienkiewicz et al. 1994). The effects of ultrasound exposure on the hippocampus and the underlying mechanisms require comprehensive exploration. Converging evidence suggests that *N*-methyl-D-aspartate (NMDA) receptor units 1 (NR1) and 2B (NR2B) and brain-derived neurotrophic factor (BDNF) play important roles in forming and maintaining hippocampus-dependent learning and memory (Tsien et al. 1996; Yamada and Nabeshima 2003), as well as normal synaptic morphology. However, there are no reports indicating that alterations in the hippocampal receptors are related to behavioral changes induced by *in utero* exposure to ultrasound. To provide experimental information about the tolerability of ultrasound during pregnancy, we investigated alterations in hippocampus-dependent spatial learning and memory, expression of NR1, NR2B and BDNF in the hippocampus of rats and synaptic structure in the offspring of pregnant rats exposed to different intensities of ultrasound.

## METHODS

### *Animals*

Male and female Sprague–Dawley rats weighing 220–250 g (Shanghai Laboratory Animal Center of the Chinese Academy of Science, Shanghai, China) were acclimated for 1 wk under a 12:12 reversed light/dark cycle (lights off at 8 AM); food and water was provided *ad libitum*. Animal care and experimental procedures were conducted in accordance with guidelines from the Chinese Animal Welfare Agency, and the study was approved by the Shanghai Ethics Committee. Rats were placed in the same cage at 19:00 every day in a male/female ratio of 1:1 and checked for pregnancy at 07:00 the next morning using the vaginal plug method. The day of pregnancy determination was defined as day 0 of pregnancy and was used to determine gestational age. Thirty rats were confirmed as pregnant and were randomly divided into three groups according to the duration of ultrasound exposure: pseudo-exposure group (control group), 4-min exposure group (4-min group) and 20-min exposure group (20-min group).

### *Ultrasound exposure*

For ultrasound exposure, we used a Philips HD7 color Doppler diagnostic ultrasound system (Philips Healthcare, Andover, MA, USA). An "Obstetrics" application setting of B-mode was used in this study; the frequency was 3.5 MHz, with a focal depth of 20 mm. The detailed irradiation conditions were modified for the treatment groups. In the 20-min group, the spatial peak time average intensity ($I_{SPTA}$) was 106 mW/cm$^2$, the mechanical index (MI) was 1.4 and the thermal index bone (TIB) was 1.0, which represented routine conditions in human medical clinics. In the 4-min group, the $I_{SPTA}$ was 7.6 mW/cm$^2$, the MI was 0.1 and the TIB was 0.1, which represented the minimum output power required for a clear picture (Philips Healthcare). The pregnant rats were exposed to these ultrasound conditions on days 6, 12 and 18 of pregnancy; these time points are equivalent to the early, middle and late phases of human pregnancy and were similar in frequency to clinical prenatal ultrasounds. The pregnant rats were anesthetized with an intraperitoneal injection of 0.3% pentobarbital sodium (30 mg/kg) and fixed on the experimental table. The lower abdomen was smeared with coupling gel; the ultrasound transducer was handled manually and kept in constant motion for the entire duration so as to ensure sonication of the entire abdominal area. Pseudo-scanning was performed in the sham control group. At 8 wk of age, 40 offspring rats (20 males and 20 females) were randomly selected from each group, categorized in the same group as their parents and subjected to behavioral testing and other experiments (Fig. 1). The experiments were performed blind to exposure status.

### *Morris water maze experiment*

The Morris water maze (MWM; Shanghai Mobile Datum Information Technology, Shanghai China) was used to assess learning and memory in the 8-wk-old offspring rats. A black fiberglass tank, 180 cm in diameter and 55 cm deep, was filled with water to a depth of 42 cm. The water was kept at a constant temperature throughout the experiment (22.0 ± 2.0°C). The water pool was divided into four equal quadrants. In the third quadrant, a Plexiglas platform was submerged 2 cm below the surface of the water. A video camera mounted above the maze was connected to a computer tracking system to record the swim pattern and escape latency.

The place-navigation experiment lasted 4 d. Each day, a quadrant was selected randomly from the point at which the rat was placed along the wall into the water. The latency referred to the time from when the rat was placed in the water to the time it found the submerged platform. The maximum latency allowed was 90 s, and the rat was required to stay on the platform for more than 5 s. If the rat failed to find the platform within 90 s, it was led to the platform and required to stay there for 5 s. The latency in these rats was recorded as 90 s. The intertrial interval was 30 s. Each group (n = 16)