Original Article

Damage to Hippocampus of Rats after Being Exposed to Infrasound

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Abstract

Objective The objective was to observe damage of hippocampus in rats after exposure to infrasound, and to assess HSP70 expression in hippocampus.

Methods SD rats in the experimental group were exposed to 140 dB (8 Hz) infrasound for 2 h per day for 3 days. The morphology of the hippocampus was examined by transmission electronic microscopic (TEM). Cell apoptosis was observed by TUNEL staining at 0 h, 24 h, 48 h, and 2 w after exposure. HSP70 expression was detected by immunohistochemistry (IHC) and Western blotting (WB).

Results TEM showed that hippocampus was significantly damaged by exposure, and exhibited recovery 1 week after exposure. The TUNEL data showed that neuronal apoptosis after exposure was significantly higher than in the control rats at 24 h and 48 h, and the apoptotic cells decreased one week after exposure. IHC and WB showed HSP70 expression was significantly higher in the exposed rats, peaked at 24 h.

Conclusion Exposure to 140 dB (8 Hz) infrasound for 2 h per day for 3 days appeared to induce damage to the hippocampus of rats, based on changes in ultrastructure and increased cell apoptosis. However, recovery from the damage occurred overtime. HSP70 expression also increased after the exposure and decreased by 48 h.

Key words: Infrasound; Hippocampus; Apoptosis; HSP70

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INTRODUCTION

Infrasound is acoustic energy with frequencies up to 20 Hertz, which is extensively emitted into the surrounding environment by industrial manufacturing, natural events, and even the human body[1-2]. Currently, people are paying increasing attention to infrasound as one of the most important contributors to environmental pollution[3]. Each part of the human body always vibrates in a specific rhythmic, with a frequency that is between 2 and 16 Hz, which is almost the exact range of infrasound. Therefore, the human may be damaged by infrasound.

Previous research showed that the brain is one of the most sensitive target organs of infrasound[4]. Infrasound of specific parameters may cause changes in learning and memory function, as well as

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the structure of the brain[5-6]. Research also demonstrated that the apoptosis of hippocampal cells was closely related to learning and memory disabilities[7]. However, most previous studies have focused on biological changes caused by continuous electromagnetic waves which may last for weeks even months[8-9]. During preliminary testing, we observed that rats exposed to infrasound at 140 dB (8 Hz) for 2 h per day for 3 days exhibited longer escape latencies and less time staying in quadrant *P* of the Morris Water Maze (MWM). In this study, we applied infrasound at 140 dB (8 Hz) for 2 h per day for 3 days to study morphological changes in the hippocampus and the apoptosis of hippocampus cells for the first time, and the mechanism through which learning and memory disabilities are caused by infrasound.

The heat shock proteins (HSPs) are a family of conserved and ubiquitously expressed proteins. The HSP70s are important for main taining the body's steady state, and help to protect cells from stress[10-11]. HSP70 proteins also protect cells from thermal and oxidative stress and other stresses. These stresses normally lead to protein damage, for instance, partial unfolding, and even aggregation. HSP70s prevent partially denatured proteins from aggregating, and allow them to refold by temporarily binding to hydrophobic residues produced by stress[12]. HSP70s also seem to participate in the disposal of damaged or defective proteins. Finally, HSP70s directly inhibit apoptosis by blocking the recruitment of the key proteins in the apoptosis pathway[13].

The expression of HSP70 in the central nervous system is a sign of neuronal excitation. Normally, brain tissue expresses only a small amount of HSP70[14]. However, when tissue is damaged, the proteins secreted by the damaged cells will induce an increased expression of HSP70, which can protect neurons[15]. Hence, the study of the expression of HSP70 in the hippocampus of rats damaged by exposure to infrasound, may lead to potential treatments for diseases caused by infrasound.

MATERIALS AND METHODS

Infrasound Exposure Apparatus

The experimental infrasound system consisted of an air compressor, an air modulator, an infrasound tank, and a monitoring platform. The infrasound tank was equipped with alight, temperature sensor, humidity sensor, infrasound microphones, and two cameras. The acoustic signal and the responses of the rats were detected by the infrasound microphones and cameras. The monitoring platform controlled the infrasound frequency and analyzed the acoustic signals received by the microphone, and modulated the sound intensity and the spectral characteristics. The experimental infrasound frequency was 8 Hz, the sound pressure level was 140 dB, and exposure time was 2 h once a day for 3 days in the study.

Animals and Experimental Groups

The experimental protocol used in this study was approved by the Ethics Committee for Animal Experimentation of the Fourth Military Medical University and the study was conducted according to the Guidelines for Animal Experimentation of the Fourth Military Medical University (Xi'an, Shaanxi, China). Male Sprague-Dawley (SD) rats weighing 100-110 g were obtained from the Animal Center of the Fourth Military Medical University (Xi'an, China). The animals were housed in stainless-steel cages in a temperature-controlled room, with a 12/12 light/dark cycle, and were allowed free access to semi-purified rat chow and pre-prepared drinking water.

Healthy male SD rats were divided into a sham group and an exposed group, which was exposed to 140 dB (8 Hz) infrasound for 2 h per day for 3 days. Before exposure, the rats were adapted to the environment of the infrasound tank. The observation points were 0 h, 24 h, 48 h, 72 h, 1 w, 2 w, and 4 w after exposure to the infrasound. Each observation points contained included 18 rats, 6 for HSP-70 immunohistochemistry and TUNEL staining, 6 for HSP-70 Western Blotting, and 6 for the TEM protocol.

TEM Examination of the Hippocampus of the Rats

A transmission electronic microscopic (TEM) examination was conducted at the Center Laboratory of the Fourth Military Medical University, using a JEM-100SX electronic microscope (Hitachi, Tokyo, Japan). At 0 h, 24 h, 48 h, 72 h, 1 w, 2 w, and 4 w after infrasound exposure, 6 animals from the sham group or the exposed group were anaesthetized with 60 mg/kg sodium pentobarbital, IP. The heart was exposed and the left ventricle was perfused with 0.9% saline, followed by perfusion for 2 h with a fixative consisting of one part 4% lanthanum nitrate and two parts 6% glutaraldehyde in 0.1 mol/L sodium cacodylate (pH 7.40-7.50). At the end of the brain perfusion, the hippocampus was