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INVOLVEMENT OF MICROGLIAL CELLS IN INFRASONIC NOISE-INDUCED STRESS VIA UPREGULATED EXPRESSION OF CORTICOTROPHIN RELEASING HORMONE TYPE 1 RECEPTOR

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Abstract—Infrasound is a kind of environmental noise and threatens the public health as a nonspecific biological stressor. Upregulated expression of corticotrophin releasing hormone (CRH) and its receptor CRH-R1 in the neurons of hypothalamic paraventricular nucleus (PVN) was reported to be responsible for infrasonic noise-induced stress and injuries. Recent studies revealed that CRH-R1 is expressed in activated microglial cells, lending support to the hypothesis that microglial cells may be also responsible for infrasonic noise-induced stress. In this work, we exposed Sprague–Dawley rats and *in vitro* cultured microglial cells to infrasound with a main frequency of 16 Hz and a sound pressure level of 130 dB for 2 h, and examined the changes in the expression of CRH-R1 at different time points after infrasound exposure by immunohistochemistry and semi-quantitative RT-PCR. We found that infrasound exposure resulted in a significant activation of microglia cells and upregulated their expression of CRH-R1 in the PVN *in vivo*. Upregulated expression of CRH-R1 can be blocked by antalarmin, a selective CRH-R1 antagonist. Our *in vitro* data further revealed that in the absence of neurons, infrasound can directly induce microglial activation and upregulate their CRH-R1 expression. These findings suggest that in addition to the PVN neurons, microglial cells are the effector cells for infrasound as well, and involve in the infrasound-induced stress through upregulated expression of CRH-R1.

Key words: infrasound, microglial cells, CRH-R1, CRH, PVN.

Infrasonic noise refers to acoustic oscillation with a frequency below 20 Hz, which is hard to detect by the human ear. Due to its characteristics of strong vibration/penetration, low attenuation during long distance propagation, and difficulty in protection, infrasound has become a new public health hazard (Backteman et al., 1984; Arabadzhi, 1992). Accumulating evidence has revealed the adverse effects of infrasound on humans and rodents (Dadali et al., 1992; Wei et al., 2002; Shi et al., 2003; Wang et al., 2005; Pei et al., 2007, 2009). As a nonspecific biological stressor, infrasound can affect the entire body via both the autonomic nervous system and the neuroendocrine system (Shiraishi et al., 1990; Yamasumi et al., 1994; Morell et al., 1997). The hypothalamic–pituitary–adrenal (HPA) axis is a major part of the neuroendocrine system that controls stress response. Our previous study showed that exposure to 8 Hz, 120 dB infrasound for 2 h significantly upregulated the expression of the corticotrophin-releasing hormone (CRH) in the rat brain (Han et al., 1999), indicating a molecular mechanism underlying infrasound-induced stress. CRH, a central regulator of the hormonal stress response, is secreted from neurons in the hypothalamic paraventricular nucleus (PVN) and exerts its function through binding to CRH type 1 receptor (CRH-R1), subsequently stimulating the secretion of corticotrophin and glucocorticoid (Shiraishi et al., 1990; Yamasumi et al., 1994; Chalmers et al., 1996). Though CRH-expressing neurons in the PVN are generally considered the main cell population involved in stress response, our previous study showed that repeated infrasound exposure resulted in an overt activation of microglial cells (Xu et al., 2008), suggesting that in addition to neurons, microglial cells are likely to be the effector cells for infrasound as well.

Microglial cells represent the immune system in the mammalian brain parenchyma and are critically involved in various injuries and diseases of the CNS. In normal brain, microglial cells are in a resting state, with characteristic small cell bodies and highly-ramified cellular processes (Davalos et al., 2005; Nimmerjahn et al., 2005). Resting microglial cells are thought to functionally interact with neurons, astrocytes and vascular endothelial cells through their processes (Nimmerjahn et al., 2005). Any insults or diseases can activate microglial cells, which undergo rapid morphological changes, such as having enlarged cell bodies, becoming ameba-shaped, and possessing enhanced phagocytic function. In addition, activated microglial cells release certain cytokines, such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-10, becoming either reparative or harmful (Garden and Moller, 2006) for the injured brain. A recent *in vitro* study revealed that activated microglial cells induced by endotoxin or hypoxia expressed high levels of CRH-R1 (Wang et al., 2002; Stevens et al., 2003), which is usually thought to be expressed in neurons. Moreover, addition of exogenous CRH to cultured microglial cells promoted their proliferation and release of TNF-α, which can be blocked by antalarmin, a CRH-R1 antagonist (Wang et al., 2003), suggesting a possible role of microglial cells in stress response.

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Abbreviations: CRH, corticotrophin releasing hormone; CRH-R1, corticotrophin releasing hormone type 1 receptor; IL, interleukin; NO, nitric oxide; PVN, hypothalamic paraventricular nucleus; TNF-α, tumor necrosis factor-α.

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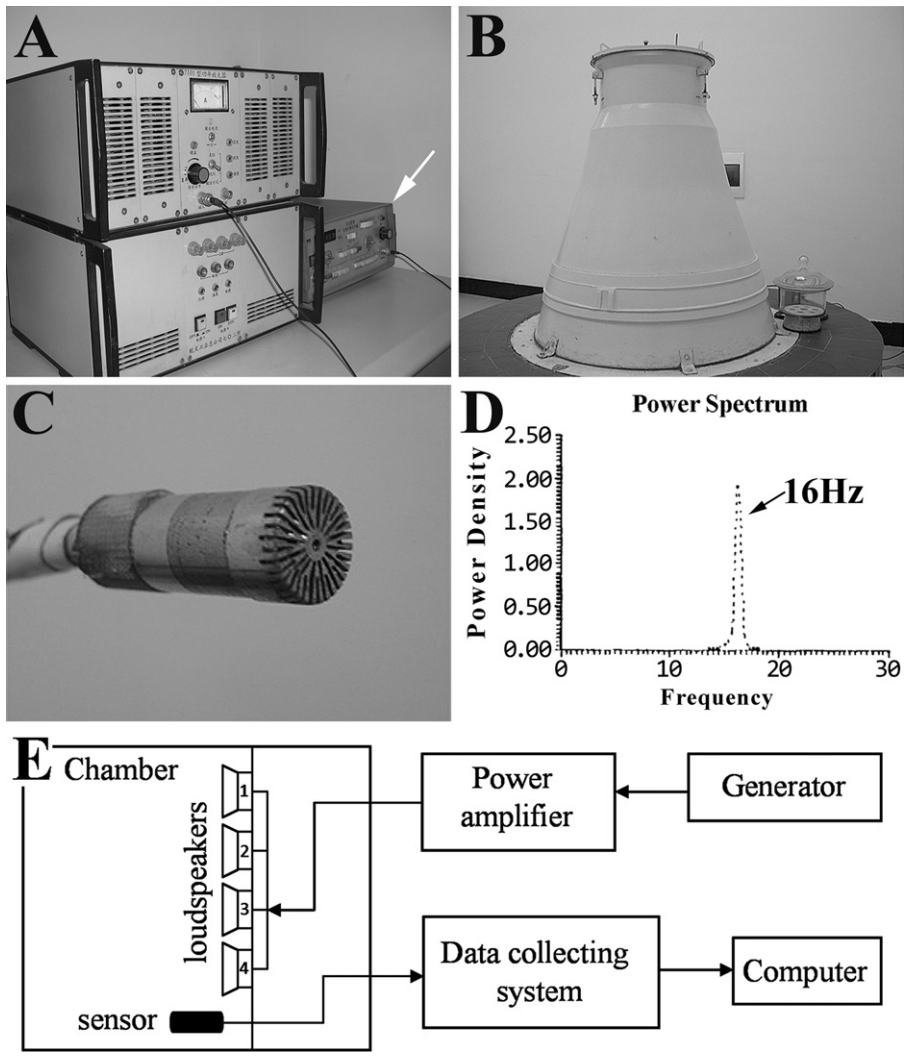

Fig. 1. The infrasonic pressure chamber system. (A) shows the low-frequency signal generator (arrow) and the power amplifier. (B) shows the infrasonic chamber with four loudspeakers in it. (C) shows the infrasonic sensor which is connected with the data collection system. (D) A 16 Hz frequency output from the low-frequency signal generator was displayed on the computer via the sensor and data collection system. (E) shows the flowchart of the infrasonic pressure chamber system.

Given that microglial cells are activated under infrasonic noise-induced stress (Xu et al., 2008), we hypothesized that, in addition to CRH-expressing neurons, microglial cells are likely to be responsible for infrasound-induce stress via CRH-R1 expression as well. In the present study, we exposed the rats and cultured microglial cells to 16 Hz, 130 dB infrasound for 2 h, and observed dynamic changes in the expression of CRH-R1 in microglial cells at different time points after infrasound exposure in the PVN. Our data showed that infrasound exposure induced significant microglial activation and upregulation of CRH-R1 expression in microglial cells, suggesting a novel mechanism whereby microglial cells are involved in infrasound-induced stress.

EXPERIMENTAL PROCEDURES

Infrasound device

The infrasound device in the present study includes a low-frequency signal generator (1110B, Beijing Intensity Environment Institute, Beijing, China) with a power amplifier (No.7101, 702 Institute of Spaceflight Co., Beijing, China) (Fig. 1A), a chamber containing four loudspeakers (YD500-8XA, Nanjing Electroacoustic Equipment Co., Nanjing, Jiangsu, China) (Fig. 1B), an infrasonic sensor (ACO Pacific, Belmont, CA, USA) (Fig. 1C) and a data collection system. The chamber covers an area of 1.96 m^2 with an effective space of 1.92 m^3. To generate infrasound, the output of the low-frequency signal generator is amplified by the power amplifier, which is then fed to the loudspeakers in the chamber. The frequency and sound pressure level of infrasound in the chamber are monitored by a sensor connected to the infrasonic data collection system and displayed on the computer (Fig. 1E). The infrasonic chamber system can generate standard infrasonic waves with a frequency range from 2 to 20 Hz and a sound pressure level from 90 to 140 dB. According to our previous studies (Wei et al., 2002; Shi et al., 2003; Wang et al., 2005), infrasound with a frequency of 16 Hz and a pressure level of 130 dB (Fig. 1D) was used in the study. The frequency and pressure level were held steady during 2 h of animal exposure and monitored by the data collection system.