The hippocampus may be more susceptible to environmental noise than the auditory cortex

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Abstract

Noise exposure can cause structural and functional problem in the auditory cortex (AC) and hippocampus, the two brain regions in the auditory and non-auditory systems respectively. The aim of the present study was to explore which one of these two brain regions may be more susceptible to environmental noise. The AC and hippocampus of mice were separated following 1 or 3 weeks exposure to moderate noise (80 dB SPL, 2 h/day). The levels of oxidative stress and tau phosphorylation were then measured to evaluate the effects by noise. Results showed significant peroxidation and tau hyperphosphorylation in the hippocampus with 1 week of noise exposure. However, the AC did not show significant changes until exposure for 3 weeks. These data suggest that although the hippocampus and AC were affected by moderate noise exposure, the hippocampus in the non-auditory system may have been more vulnerable to environmental noise than the AC.

Key words: noise exposure, hippocampus, auditory cortex, oxidation, tau phosphorylation

1. Introduction

Noise pollution has become a serious social and public health problem for human beings (Ising and Kruppa, 2004; Goines and Hagler, 2007). Physiological structures and functions of several systems especially the auditory system, can be affected by exposure to environmental noise (Eggermont, 2014, 2015; Stamper and Johnson, 2015). In the auditory system,

environmental noise induces cell death (Basta, et al., 2005) and a threshold shift (Syka and Rybalko, 2000), and causes abnormal neural coding (Willott and Lu, 1982; Pienkowski and Eggermont, 2010, 2012) in the auditory cortex (AC) and other cortical regions responsible for acoustic processing (Syka and Popelář, 1982). In contrast, environmental noise also affects non-auditory brain regions such as the hippocampus, a major limbic region that receives direct or indirect neural input from the central auditory system (Rauschecker, et al., 2010; Kraus and Canlon, 2012). Accumulating evidence illustrates a significant effect of strong noise on hippocampal-related cognition (Kim, et al., 2006; Manikandan, et al., 2006). A number of studies including our own have highlighted that exposure to strong or moderate noise can affect the physiological structure and subsequently the function of both the AC and hippocampus (Willott and Lu, 1982; Kim, et al., 2006; Cheng, et al., 2011). However, does the AC, the primary brain region to process acoustic information, have high susceptibility to environmental noise than the hippocampus in non-auditory system? It has not been determined.

Previous studies have shown that as a stressor, noise may cause extensive oxidative stress along the lemniscal ascending pathway including the AC and the hippocampus (Manikandan, et al., 2006; Cheng, et al., 2011). In organisms, peroxidation induces malondialdehyde (MDA) content increase, which causes cellular toxicity, and in response, superoxide dismutase (SOD) is produced as an antioxidant defense. There is also evidence that peroxidative stress induces tau hyperphosphorylation, which can destroy the morphological structure and alter the normal physiological function of neurons (Grundke-Iqbal, et al., 1986; Su, et al., 2010). Therefore, in the present study, we sought to

determine which of the two brain regions (the AC and hippocampus) is more susceptible to environment noise by measuring peroxidation levels (i.e., the MDA content and SOD activity) and tau phosphorylation in these two regions of mice following noise exposure. We have previously shown that a long-term noise exposure (6 weeks) cause both the AC and hippocampus affected (Cheng, et al., 2011). Therefore, in order to check which one of these two regions would be affected easier, mice in the present study were exposed for a short-term duration (1 week and 3 weeks). A moderate level of noise was used to avoid direct physical damage.

2. Materials and methods

2.1 Animals and noise exposure

All 3-week-old kunming mice (*Mus musculus*, Km) (n=44, 9-12 g, SPF) used in this study were purchased from the Center for Disease Control and Prevention of Hubei province of China. Animals were housed in plastic cages on natural light cycles with free access to food and water. The ambient temperature was maintained at 20-25 °C. All experiments were approved by the Institutional Animal Care and Use Committee of Central China Normal University and complied with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Animals were divided randomly into four groups: 1 week of noise (n=11) and control (n=11), and 3 weeks of noise (n=11) and control (n=11). As the different noise exposure time between the two test groups resulted in an age disparity, an age-matched control group for each test group was required. Following noise exposure for 1 and 3 weeks, mice were used to examine the peroxidation levels and the level of

phosphorylation of tau in the AC and hippocampus, respectively.

The continuous white noise used in this study was generated by a noise generator (Nanjing University, Nanjing, Jiangsu, China) and amplified by a power amplifier (custom-made amplifier) before being delivered to mice housed in cages (one mouse per cage). The amplitude of the noise was calibrated to 80 dB SPL, and the range of noise frequency was from 10 to 10000 Hz. For the two noise groups, the noise exposure was performed for 2 hours per day continuously and randomly.

2.2 Antibodies and chemicals

Monoclonal antibodies (mAb) Tau-5 that recognizes total tau was purchased from Invitrogen Inc. (Carlsbad, CA, USA). The rabbit polyclonal antibody (pAb) Ps396 that recognizes phosphorylated tau at Ser396 epitope was purchased from Biosource Inc. (Carmarillo, CA, USA). The mAb Tau-1 that recognizes unphosphorylated tau at Ser195/198/199/202 epitope was purchased from Millipore Corporation (Bedford, MA, USA). Horseradish peroxidase-conjugated secondary antibodies were from Pierce Chemical Co. (Rockford, IL, USA). The chemiluminescent substrate kit was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). MDA content and SOD activity were detected using MDA and SOD assay kits, respectively (Jiancheng, Nanjing, Jiangsu, China). The bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA) was used to measure protein concentration. Other routine chemicals were from Sinopharm Chemical Reagent Co. (Shanghai, China).

2.3 Measurement of MDA and SOD

Following the noise exposure for 1 and 3 weeks, 6 mice from each group (24 in total) were selected randomly and sacrificed. The brains were quickly removed, and the whole AC and hippocampus were separated out on ice according to their locations: AC, on the caudal half of the parietal cortex, nearly 1.0 mm from the occipital end of the cortex and 3.0 mm from the sinus petrosus, about 2.0 mm × 2.3 mm in size (Stiebler, et al., 1997); hippocampus, a crescent-shaped structure beneath the caudal half of the parietal cortex, 1.0–4.0 mm from bregma, 0.5–3.5 mm from the sinus petrosus (Paxinos and Franklin, 1997). Then, they were ground into a 10% tissue homogenate with 0.1 M PBS buffer solution (pH 7.4). The tissue homogenates were centrifuged at 10000×g at 4 °C for 10 min, and the supernatants were collected and prepared for measurement of MDA content and SOD activity according to the manufacturer's description.

2.4 Western blotting

Following the noise exposure for 1 and 3 weeks, 5 mice in each group (20 in total) were used to measure the level of phosphorylation of tau in the AC and hippocampus. Mice were sacrificed, and the AC and the hippocampus were removed (see part 2.3 for details) and homogenized in buffer containing 10 mM Tris-Cl (pH 7.6), 50 mM NaF, 1 mM Na₃VO₄, 1 mM edetic acid, 1 mM benzamidine, 1 mM PMSF, and a mixture of aprotinin, leupeptin and pepstain A (10 µg/ml each). After measurement of protein concentration in the supernatant of the homogenate using the BCA Kit, a final concentration of 10% β-mercaptoethanol and 0.05% bromophenol blue were added and the samples were boiled for 10 min. Equal amounts

of proteins were isolated on 10% SDS-polyacrylamide gels (SDS-PAGE) and transferred to nitrocellulose membranes (Hybond C-Super, Amersham Pharmacia Biotech, Amersham, UK). The membranes were blocked in 5% (w/v) nonfat milk in TTBS (10 mM Tris-HCl, 150 mM NaCl, 0.02% (v/v) Tween-20, pH 7.5) and probed with Tau-1 (1:1000), Tau-5 (1:1000), or PS396 (1:1000) overnight at 4 °C. The blots were developed with horseradish peroxidase-conjugated secondary antibodies (1:4000) and visualized by enhanced chemiluminescent substrate kit and exposure to CL-XPosure film. Tau immunoreactivity was quantitatively analyzed by Kodak Digital Science 1D software (Eastman Kodak, New Haven, CT, USA) and expressed as mean optical density. The levels of total and phosphorylated tau at various epitopes were expressed as the relative level of the mean optical density against control, and the phosphorylated level of tau was normalized to total tau.

2.5 Statistical analysis

Data were analyzed and plotted with SPSS 13.0 and Sigmaplot 10.0. Independent *t*-test was used to determine the statistical significance.

3. Results

3.1 Noise-induced peroxidation levels in the AC and hippocampus

A 1-week exposure of noise significantly increased the content of MDA and markedly decreased SOD activity in the hippocampus ($P < 0.01$, Fig. 1A). No changes were observed in the AC (Fig. 2A). However, exposure to noise for 3 weeks increased the content of MDA and decreased SOD activity in both brain regions ($P < 0.05$ or 0.01 , Fig. 1B and 2B). Therefore,

these findings showed that noise exposure for 1 week induced hyperoxidation in the hippocampus, and hyperoxidation in the AC occurred only with a 3-week exposure.

3.2 Noise-induced levels of tau hyperphosphorylation in the AC and hippocampus

Because tau protein phosphorylation has been associated with peroxidative stress, we investigated the phosphorylation level of tau protein in the AC and hippocampus. Exposure to noise for 1 week significantly decreased and increased the immunoreactivity of non-phosphorylated tau at Ser195/198/199/202 sites (probed by Tau-1) and phosphorylated tau at Ser396 epitope (probed by Ps396), respectively, in the hippocampus ($P < 0.01$, Fig. 3A and B). No changes were found in the AC (Fig. 4A and B). However, noise exposure for 3 weeks significantly decreased and increased non-phosphorylated and phosphorylated tau, respectively in the hippocampus ($P < 0.01$; Fig. 3A and C) and the AC ($P < 0.01$; Fig. 4A and C). No significant changes were observed in total tau (probed by Tau-5) for either exposure time. This results showed 1-week exposure of noise could induce tau hyperphosphorylation in the hippocampus, but not in the AC until 3-week exposure.

4. Discussion

The central nervous system undergoes progressive structural and functional maturation during postnatal development, and noise exposure, as a stressor, may therefore affect brain function of mice to a greater extent at this stage than in adults (Freeman, et al., 1999; Rybalko and Syka, et al., 2001; Chang and Merzenich, 2003). Therefore, young mice were used in the current study. Our data demonstrated that 1-week exposure of noise increased oxidation and

tau phosphorylation in the hippocampus of young mice (Fig. 1A, 3A, B). However, the AC did not show significant changes until a 3-week exposure (Fig. 2, 4). These findings suggest that the hippocampus in the non-auditory system may be more vulnerable to environmental noise exposure than the AC, although both the hippocampus and the AC have been shown to be affected by noise exposure (Fig. 1B, 2B, 3C, 4C).

In the present study, mice were exposed to moderate noise to avoid direct physical damage (Freeman, et al., 1999; Cappaert, et al., 2000). As such, indirect effects by noise, such as emotional stress, would be the main effects, especially in 1-week exposure group. Therefore, the hippocampus, as a brain region related to emotional stress, may be more easily affected by noise exposure than the AC (Adhikari, et al., 2010; Leal, et al., 2014). Besides inducing stress, noise, as an acoustic stimulus, would probably produce abnormality in the AC and hippocampus through inducing their functional changes (Goble, et al., 2009; Zheng, 2012). But the early effects in hippocampus reported in the present study are probably not due to acoustic input from cortex to the hippocampus. Because if so, the AC, as a primary brain region of acoustic processing, should be affected first. But actually, the moderate noise has earlier effect on the hippocampus. So, the observed changes in the present study are primarily attributed to the stress induced by noise, rather than the noise as an acoustic stimulus. Future study will be needed to test the prediction.

The hippocampus and the AC are critical for learning and memory and auditory functions, respectively (Nourski and Brugge, 2011; Cheng, et al., 2014). Therefore, peroxidation and hyperphosphorylation observed in the hippocampus and the AC may play a role in cognitive and hearing deficits. Based on our data, the hippocampus may be more

susceptible to noise than the AC, and thus hippocampal-related cognition may be primarily affected by noise exposure more than hearing.

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Figure legends

Fig. 1. Changes in the content of malondialdehyde (MDA) and superoxide dismutase (SOD) activity in the hippocampus of mice. MDA content and SOD activity in the hippocampus of mice following (A) 1 or (B) 3 weeks of noise exposure. Con, control group; Noi, noise group; 1 w, noise exposure for 1 week; 3 w, noise exposure for 3 weeks. Independent *t*-test, $**P < 0.01$ vs. Control.

Fig. 2. Changes in the content of MDA and SOD activity in the auditory cortex (AC) of

mice. MDA content and SOD activity in the AC following (A) 1 or (B) 3 weeks of noise exposure. Con, control group; Noi, noise group; 1 w, noise exposure for 1 week; 3 w, noise exposure for 3 weeks. Independent *t*-test, **P* < 0.05, ***P* < 0.01 vs. Control.

Fig. 3. Immunoreactivity of tau protein in the hippocampus of mice following 1 or 3 weeks of noise exposure. (A) Representative immunoblots of proteins extracted from the hippocampi of control mice and mice exposed to 1 or 3 weeks of noise and probed with the following primary antibodies: Tau-5, Tau-1, or phosphorylated (p)-Ser396. Density analysis in (B) a 1-week noise exposure and (C) 3-weeks exposure to show the levels of total tau (probed by Tau-5 antibody), non-phosphorylated tau (probed by Tau-1 antibody at Ser195/198/199/202 sites) and p-tau (probed by p-S396 antibody at Ser 396 epitope) in the hippocampus of mice. Data were normalized to the level of tau protein in the control group. Protein levels are expressed as fold changes over control and represent the mean $\pm$ SD of three independent trials. Independent *t*-test, ***P* < 0.01 vs. control.

Fig. 4. Immunoreactivity of tau protein in the AC of mice following 1 or 3 weeks of noise exposure. (A) Representative immunoblots of proteins extracted from the AC of control mice and mice exposed to 1 or 3 weeks of noise and probed with the following primary antibodies: Tau-5, Tau-1, or phosphorylated (p)-Ser396. Density analysis in (B) a 1-week noise exposure and (C) 3-weeks exposure to show the levels of total tau (probed by Tau-5 antibody), non-phosphorylated tau (probed by Tau-1 antibody at Ser195/198/199/202 sites) and p-tau (probed by p-S396 antibody at Ser 396 epitope) in the AC of mice. Data were normalized to

the level of tau protein in the control group. Protein levels are expressed as fold changes over control and represent the mean $\pm$ SD of three independent trials. Independent *t*-test, ***P* < 0.01 vs. control.

Figure 1

Figure 2

Figure 3

Figure 4