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NOISE EXPOSURE–INDUCED ENHANCEMENT OF AUDITORY CORTEX RESPONSE AND CHANGES IN GENE EXPRESSION

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Abstract—Noise exposure is one of the most common causes of hearing loss. There is growing evidence suggesting that noise-induced peripheral hearing loss can also induce functional changes in the central auditory system. However, the physiological and biological changes in the central auditory system induced by noise exposure are poorly understood. To address these issues, neurophysiological recordings were made from the auditory cortex (AC) of awake rats using chronically implanted electrodes before and after acoustic overstimulation. In addition, focused gene microarrays and quantitative real-time polymerase chain reaction were used to identify changes in gene expression in the AC. Monaural noise exposure (120 dB sound pressure level, 1 h) significantly elevated hearing threshold on the exposed ear and induced a transient enhancement on the AC response amplitude 4 h after the noise exposure recorded from the unexposed ear. This increase of the cortical neural response amplitude was associated with an upregulation of genes encoding heat shock protein (HSP) 27 kDa and 70 kDa after several hours of the noise exposure. These results suggest that noise exposure can induce a fast physiological change in the AC which may be related to the changes of HSP expressions. Published by Elsevier Ltd on behalf of IBRO.

Key words: auditory cortex, gene expression, heat shock protein, noise exposure, gene microarray, tinnitus.

Noise exposure not only results in temporary or permanent hearing loss, but can also induce transient or permanent tinnitus, loudness recruitment and hyperacusis (Axelsson and Hamernik, 1987; Moller, 2007). Previous physiological studies suggest that the reduced neural output from the damaged cochlea alters the central auditory system by increasing its gain and possibly contributing to tinnitus, loudness recruitment and hyperacusis (Salvi et al., 2000; Wang et al., 2002). In addition, human brain imaging studies found changes in neural activity in the auditory cortex (AC) that were closely linked to changes in the loudness of tinnitus (Lockwood et al., 1998; Reyes et al., 2002). Moreover, sound stimulation evoked greater activity in the AC of patients with tinnitus and cochlear hearing loss (Lockwood et al., 1998). Similarly, in animal studies, a moderate high-frequency noise exposure can induce a significant increase of evoked potential amplitude and spontaneous neural activities (Popelar et al., 1987; Komiya and Eggermont, 2000). However, the molecular mechanisms that give rise to these changes in neural activity of the central auditory system are not clear. In order to elucidate the early molecular mechanisms associated with noise-induced hearing loss and cortical hyperactivity, we used focused gene microarrays and quantitative real time polymerase chain reaction (PCR) to identify the changes in gene expression that occur in the rats AC following a monaural noise exposure similar to that used in behavioral studies of noise-induced tinnitus used in our and other laboratories (Brozoski et al., 2007).

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Abbreviations: ABR, auditory brainstem response; AC, auditory cortex; CDKN-1A, cyclin-dependent kinase inhibitor 1A; C_T, cycle threshold; CTSD, cathepsin D; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSP, heat shock protein; IC, inferior colliculus; PCR, polymerase chain reaction; SPL, sound pressure level; TDT, Tucker-Davis Technologies.

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EXPERIMENTAL PROCEDURES

Animals

Adult Harlan Sprague–Dawley male rats (3–5 months old) weighing from 200 to 400 g were used for all studies. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University at Buffalo and conformed with the guidelines issued by the National Institutes of Health. This research minimized the number of animals used and their suffering.

Surgery procedures and evoked potential recordings

The rats used for the neurophysiological recordings were implanted with a chronic electrode in the left AC using procedures described in our previous publication (Yang et al., 2007). Rats were anesthetized with 1–2% isoflurane. The left AC was surgically exposed using anatomical locations and landmarks on the skull and blood vessels on the surface of the cortex (Sally and Kelly, 1988; Polley et al., 2007). The dura mater was excised and then a silver ball electrode made from Teflon-coated wire (0.008-inch diameter, A-M Systems, Carlsborg, WA, USA) connected to a miniature pin connector was placed on the AC. A stainless steel electrode (0.008-inch diameter, A-M Systems) connected to a pin connector was placed on the surface of the frontal lobe and served as ground electrode. The electrode connectors were firmly attached to the skull using the cyanoacrylic glue and dental cement. Six small stainless steel screws (1/8-inch, Small Parts Inc., Miramar, FL, USA) were attached to the skull and covered with dental cement; these were used to help anchor a head restraint screw (1/2-inch long with ¼-inch diameter) to the skull using dental cement. The wound was sutured around the electrode connector and then the animal was allowed to recover for 7–10 days before the physiological test.

Sound stimuli were generated with Tucker-Davis Technologies (TDT, Alachua, FL, USA) hardware and presented through a high frequency speaker (Fostex FT28D, Madisound Speaker Components Inc., Middleton, WI, USA). Tone-bursts (10 ms du-

ration, 1 ms rise/fall time, twice per second) from 4 to 32 kHz were used to elicit the AC local field potential. Responses were amplified and filtered (10–3000 Hz) and digitized (25 kHz sampling rate, sampling duration 300 ms). For the auditory brainstem response (ABR) recording, the implanted stainless steel electrode on the surface of the frontal lobe was used as the non-inverting recording site, the ipsilateral pinna was used as the inverting recording site and contralateral pinna was used as the ground. The sound stimuli used to elicit the AC response were also used for ABR recording. The sound levels were calibrated using a sound level meter (824, ½-inch microphone; Larson Davis, Depew, NY, USA). The electrodes for the AC and ABR recordings were connected to a preamplifier (RA16LA, TDT) using a flexible, low noise cable. The output of the preamplifier was sent to a digital signal processing module (RX5-2, Pentusa Base Station, TDT) and collected by software (BioSigRP version 4.4, TDT).

Focused gene microarrays

Noise-induced changes in gene expression in the AC were evaluated with the Signal Transduction Pathway Finder Array (ORN-014, SuperArray Bioscience Corporation, Frederick, MD, USA). This gene array contains 112 genes, includes 96 genes associated with common signal transduction pathways, several housekeeping genes such as β-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cyclophilin, and ribosomal protein L13a which are relatively abundant and used as a comparison for estimating signal intensity of the target genes, and several negative controls used for quality control (Fig. 3). One group of rats (n=3) was anesthetized with isoflurane (1–2%). The left ear of each rat was exposed for 1 h to a 120 dB sound pressure level (SPL) narrow band noise (12 kHz, 1000 Hz bandwidth), while the right ear was sealed with cotton and Vaseline. The other group (n=3) was anesthetized with isoflurane (1–2%) without noise exposure and served as the control group.

Two hours after the noise exposure, rats were killed with an overdose of CO_2. The AC tissues were collected for gene array and PCR studies. The total RNA of the AC was extracted using a Total RNA Isolation kit (Z3100, Promega, Madison, WI, USA) and mRNA was reverse transcribed to cDNA using a TrueLabeling-Amp Linear RNA Amplification Kit (GA-010, SuperArray). cDNA was labeled with biotin-16-UTP (#1388908, Roche, Indianapolis, IN, USA) and transcribed to cRNA, and then purified using the cRNA Cleanup Kit (GA-012, SuperArray). The SuperArray membrane (ORN-014, SuperArray) was rinsed with hybridization solution (H-01, SuperArray) and then incubated with the biotin-labeled cRNA for 1 h in a hybridization oven. The membrane was washed twice with 5 ml washing solution and incubated for 15 min at 60 °C with agitation, blocked with GEA blocking solution for 1 h and then treated with chemiluminescence solution (D-01, SuperArray). An image of the array was captured by a Kodak image system and the density of each dot on the membrane was measured using Kodak Image software.

Quantitative real time PCR

To confirm the findings in the gene array study, quantitative real-time PCR was used to assess the changes in a subset of genes that showed a large change in expression in the gene array. The procedure used for real-time PCR and data analysis was described in our previous publication (Sun et al., 2005). Briefly, after the total RNA was extracted, the mRNA was reverse transcribed to cDNA using a First Strand Synthesis Kit (cat. 1710, Ambion, Austin, TX, USA). Five microliters of cDNA solution containing approximately 200 ng of cDNA isolated from the tissue was added to the reaction mixture (10 μl, PA-011, SuperArray) along with 5 μl of a forward and reverse primers mixture (concentration 1.25 μM) and pipetted into a well in a 96-well plate (MyIQ, BioRad, Hercules, CA, USA). Genes analyzed in this experiment included heat shock protein (HSP) 27 kDa, 70 kDa and 90 kDa, cyclin-dependent kinase inhibitor 1A (CDKN-1A) and cathepsin D (CTSD). GAPDH, an abundantly expressed housekeeping gene which was stably expressed in control and experimental groups, was used as a reference for calculating the change in expression of the target genes. Primers used for real-time PCR tests were ordered from SuperArray Company and had previously been optimized by the vendor. SYBR green fluorescence was measured during amplification and the cycle threshold (C_T) was determined when the fluorescence showed a dramatic increase above the background level. The fold changes of the target genes in the experimental group were normalized to GAPDH and the relative changes compared with the control group were calculated for each sample using the equation of $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT=(C_{T,\ Target}-C_{T,\ GAPDH})_{experiment}-(C_{T,\ Target}-C_{T,\ GAPDH})_{control}$ (Livak and Schmittgen, 2001). $C_{T,\ Target}$ represents the C_T of the target gene and $C_{T,\ GAPDH}$ represents the C_T of GAPDH. The analyses were replicated for each gene.

The graphs and statistic analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA) unless otherwise noted. Variability is indicated by the standard error of the mean.

RESULTS

ABR threshold shift

The magnitude of noise induced hearing loss was evaluated using the ABR. The average ABR thresholds on noise exposed ears before and 2 h after the noise exposure are shown in Fig. 1A. Before the noise exposure, the average ABR thresholds (mean±S.E.M., n=6) were 45±2 dB, 45±2 dB, 37±1.6 dB, 36±1.6 dB, 39±3.9 dB, 48±1.6 dB and 47±2.5 dB at 4, 6, 8, 12, 16, 24 and 32 kHz respectively. Two hours after the noise exposure, the average ABR thresholds (n=6) increased to 47±1.7 dB, 46±1 dB, 43±4 dB, 67±4 dB, 58±7 dB, 62±6 dB and 58±5 dB at 4, 6, 8, 12, 16, 24 and 32 kHz respectively. The threshold changes at 12, 16 and 24 kHz in the exposed ears were significant (paired t-test, $P<0.05$) (Fig. 1A). The unexposed ears showed less than 10 dB threshold shift (Fig. 1B). The threshold changes at 4, 6, 16, 24, and 32 kHz were not significant, however, at 8 and 12 kHz, the threshold shifts were significant (paired t-test, $P<0.05$) (Fig. 1B).

AC input/output functions

The AC local field potentials were evoked by tone-bursts centered at 6, 12, 16 and 20 kHz from 20 to 100 dB SPL. A typical AC response waveform is shown in Fig. 2A. The AC response was recorded from the left AC and sound was delivered to the right ear (non-exposed ear) on each animal. Fig. 2B–E showed typical input–output curves of the AC response measured at 6, 12, 16 and 20 kHz before and 4 h and 1 day post-exposure. Before the exposure, the AC amplitude increased monotonically above 40 dB SPL reaching its maximum amplitude around 100 dB SPL. The AC response showed a large increase 4 h after the noise exposure at sound levels above 60–70 dB SPL; however, by 1 day post-exposure the amplitudes had returned to essentially normal levels.

In order to measure the average amplitude enhancement across the entire group of animals, the post-exposure amplitude at 90 dB SPL was normalized to the pre-exposure amplitude measured at the highest intensity (100 dB