Research Report

Noise exposure enhances auditory cortex responses related to hyperacusis behavior

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ABSTRACT

Hyperacusis, a marked intolerance to normal environmental sound, is a common symptom in patients with tinnitus, Williams syndrome, autism, and other neurologic diseases. It has been suggested that an imbalance of excitation and inhibition in the central auditory system (CAS) may play an important role in hyperacusis. Recent studies found that noise exposure, one of the most common causes of hearing loss and tinnitus, can increase the auditory cortex (AC) response, presumably by increasing the gain of the AC. However, it is not clear whether the increased cortical response will affect sound sensitivity and induce hyperacusis. In this experiment, we studied the effects of noise exposure (narrow band noise, 12 kHz, 120 dB SPL, 1 hour) on the physiological response of the inferior colliculus (IC) and the AC, and the behavioral sound reaction in conscious Sprague Dawley rats. Noise exposure induced a decrease of sound evoked potential in the IC. However, significant increases of AC response including sound evoked potentials and the spike firing rates of AC neurons were recorded right after the noise exposure. These results suggest that noise exposure induces hyperexcitability of AC presynaptically increasing the post-synaptic response of AC neurons. The behavioral consequence of the noise exposure on sound perception was measured by the amplitude of the acoustic startle response in a separate group of rats. Although noise exposure caused a moderate hearing loss, the acoustic startle amplitude at the super-threshold level was significantly increased. These results suggest that noise exposure can cause exaggerated the sound reaction which may be related with the enhanced responsiveness of the AC neurons. This phenomenon may be related with noise induced hyperacusis.

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1. Introduction

Noise exposure is one of most common causes of hearing loss which may lead to various disturbing disorders, such as tinnitus, loudness recruitment and hyperacusis (Moller, 2007). The neural basis of the causes of these disorders is still largely unknown. Recent studies suggest that tinnitus and hyperacusis may be related to a functional change in...

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Abbreviations: ABR, auditory brainstem response; AC, auditory cortex; CF, characteristic frequency; GABA, γ-aminobutyric acid; IC, inferior colliculus; PSTH, peri-stimulus time histograms; rms, the root mean square

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the central auditory system (CAS) subsequent to peripheral damage (Eggermont and Roberts, 2004; Kaltenbach, 2011; Salvi et al., 2000). Our recent studies found that high doses of salicylate, a common inducer of tinnitus in both human (Atkinson et al., 1995; Proudfoot, 1983) and animals (Jastreboff et al., 1988; Lobarinas et al., 2004), can cause an enhancement of the responses in the auditory cortex (AC) (Lu et al., 2011; Sun et al., 2009; Yang et al., 2007). Since salicylate causes mild to moderate hearing loss, the increased AC response is very likely a result of increased sensitivity of AC neurons to acoustic stimuli. The increased AC response, referred to as a central gain increase, was hypothesized to be related with salicylate induced tinnitus (Eggermont and Roberts, 2004; Salvi et al., 2000; Sun et al., 2009). Interestingly, high doses of salicylate also cause enhanced acoustic startle response, indicative of hyperacusis behavior (Sun et al., 2009; Turner and Parrish, 2008; Yang et al., 2007). These results suggest that the increase in sensitivity of the CAS corresponds to sound perception changes may be related with hyperacusis.

Noise induced hearing loss is one of the most common causes of tinnitus and hyperacusis (Moller, 2007). However, in spite the substantial progress that has been made on tinnitus study, the cause of noise-induced hyperacusis is largely unknown. Similar to high doses of salicylate exposure, noise exposure can also increase AC evoked potentials in animal studies (Norena et al., 2002; Popelar et al., 1987; Sun et al., 2008; Syka, 2002). The enhanced AC response has been suggested to be related with the reduction of the central inhibition (Salvi et al., 2000). However, whether or not the increased cortical response induced by noise exposure will affect the sound reaction and hyperacusis has not been tested. In this experiment, we first record the IC and AC response from the awake rats to study the changes in the subcortical and cortical levels affected by noise exposure. Then, we tested whether noise exposure will affect the animal’s sound reaction in a separated group of rats using the acoustic startle response test, which recently has been used to measure the hyperacusis behavior in rodents (Ison et al., 2007; Turner et al., 2006; Yang et al., 2007).

2. Results

2.1. Noise exposure affects the amplitude of IC and AC response

A group of rats with chronic IC and AC electrodes implanted (n=3) were used to test the effects of noise exposure on the CAS response. A narrow band noise (120 dB SPL centered at 12 kHz with 1 kHz bandwidth for 1 hour) was used for noise exposure. Fig. 1 shows the averaged IC response (n=3) at 4, 8, 16 and 24 kHz before and 1 hour after noise exposure. At 4 and 8 kHz, the IC responses did not show any obvious changes (Fig. 1A–B). At 16 and 24 kHz, there was a 30–40 dB threshold

**Fig. 1** – The response of the inferior colliculus (IC) before and after noise exposure at (A) 4 kHz, (B) 8 kHz, (C) 16 kHz and (D) 24 kHz. The IC response showed a significant reduction 1 hour after the noise exposure at low intensity at 16 and 24 kHz, but not at 4 and 8 kHz.
shift after noise exposure; however, the amplitude of IC evoked potential at 100 dB SPL did not show a significant difference compared to the level before noise exposure (n=3, Fig. 1C–D).

The AC responses at 4 to 24 kHz before and 1 hour after noise exposure are shown in Fig. 2 (n=4). A significant increase in AC response has been shown at 16 and 24 kHz 1 hour after noise exposure (Fig. 2C–D, two-way ANOVA, p<0.05), but not at 4 or 8 kHz (Fig. 2A–B). The average AC amplitude (n=4) at 100 dB SPL increased 32±3%, 25±13%, 34±10% and 47±20% at 4, 8, 16 and 24 kHz, respectively (significant at 16 and 24 kHz, paired Student’s t-test, p<0.05; 4 and 8 kHz, not significant). The increase in AC response induced by the noise exposure recovered in 1–2 days post exposure (Fig. 3A–D).

2.2. Noise exposure on the firing rate of the AC neurons

To further test the source of the AC response enhancement, a chronic high impedance electrode was implanted in the AC in a separated group of the rats (n=9). Typical peri-stimulus time histograms (PSTH) of the AC response evoked by tone-bursts centered at the characteristic frequency (CF) of the neurons before and after noise exposure are shown in Fig. 4A–B. The average firing rates showed a significant increase at above 60 dB SPL 1 hour after the noise exposure (Fig. 4C, n=9, two-way ANOVA tests, F(1, 80)=38.63, p<0.0001). At 9 dB SPL, the averaged spike firing rate increased 84±31% (n=9) 1 hour after noise exposure and gradually dropped to the level prior to the noise exposure in 1–3 days (Fig. 4D).

2.3. Noise exposure induced hearing loss and acoustic startle amplitude changes

To test how noise exposure will affect the behavioral sound response, the acoustic startle response was measured before and after noise exposure (120 dB SPL narrow-band noise centered at 12 kHz, 1 kHz bandwidth, 1 hour) in another group of rats (n=8). The hearing thresholds were evaluated by auditory brainstem response (ABR) before and after noise exposure. One hour after noise exposure, the average hearing threshold (dB SPL) elevated from 44±6 dB, 42±6 dB, 42±7 dB and 36±7 dB to 53±20 dB, 58±19 dB, 85±6 dB and 79±11 dB at 4, 8, 16 and 24 kHz, respectively (n=8, Fig. 5A). The average threshold shift recovered 1 week after the noise exposure (Fig. 1A). Fig. 5B and C show the ABR threshold shifts of individual rats before and after noise exposure at 8 and 16 kHz. Most of the rats developed temporary hearing loss and their ABR thresholds gradually recovered in 1 week after noise exposure. However, one rat (rat #5) developed a permanent hearing loss (threshold was above 90 dB SPL) in all frequencies (4 and 24 kHz did not show). To reduce the effect of permanent hearing loss on the behavioral test, the result of this rat was eliminated from further behavioral testing analysis.
The startle amplitude–sound intensity functions at 8 and 16 kHz before and after noise exposure are shown in Fig. 6. Despite the significant elevation in ABR threshold 1 hour after noise exposure, the startle amplitude at sound intensities above their thresholds did not show an obvious reduction. In fact, at 16 kHz, the startle amplitude 1 hour after noise exposure was actually larger than the amplitude before noise exposure ($n=7$, two-way ANOVA, $p<0.05$). The startle response recovered on the second day after the noise exposure. The relative startle amplitude change at 100 dB SPL (normalized based on the average startle amplitude before the noise exposure) at 8 and 16 kHz are shown in Fig. 6C and D. The average startle amplitude showed 100% to 200% increase 1 hour after noise exposure and gradually recovered on the second day.

3. Discussion

The first interesting finding of this experiment is that the noise exposure causes increased sound evoked potential in the AC despite of the peripheral hearing loss (Syka and Rybalko, 2000). Furthermore, from a separate group of rats, we detected an increase of AC spike firing rate right after noise exposure was actually larger than the amplitude before noise exposure ($n=7$, two-way ANOVA, $p<0.05$). The startle response recovered on the second day after the noise exposure. The relative startle amplitude change at 100 dB SPL (normalized based on the average startle amplitude before the noise exposure) at 8 and 16 kHz are shown in Fig. 6C and D. The average startle amplitude showed 100% to 200% increase 1 hour after noise exposure and gradually recovered on the second day.

3.1. Noise exposure enhanced AC response

Our physiological findings on noise induced increase of AC response are consistent with the previous reports (Norena et al., 2010; Popelar et al., 1987; Sun et al., 2008; Syka and Rybalko, 2000). Furthermore, from the same animals, we found noise trauma decreased the output of the IC. These results suggest that noise exposure can directly affect the output of cortical neurons presumably through increasing the responsiveness of the AC neurons. A recent report from Norena et al. found an increase in the amplitude of local field potentials in the AC immediately after noise exposure and recovered on the second day in awake guinea pigs (Norena et al., 2002). Their results also suggest that noise exposure can enhance postsynaptic potentials coming from the thalamus toward the cortical neurons.

The cortical response was suppressed largely by the cortical inhibitory circuitry formed by GABA receptors in the thalamocortical projections and the recurrent inter-cortical inputs (Liu et al., 2007). Previous studies have shown that noise exposure can reduce the cortical inhibition (Rajan, 1998; Scholl and Wehr, 2008). Using in vivo whole cell patch-clamp recordings, Scholl and Wehr found that acute acoustic trauma abolished acoustic startle response at the super-threshold levels. This result suggests that noise exposure can cause an increased sound reaction to loud sound, which may be indicative of hyperacusis behavior (Ison et al., 2007; Sun et al., 2009; Turner and Parrish, 2008).
the cortical inhibition and led to an expansion of receptive fields within 1 hour suggesting an acute disinhibition in the cortical area (Scholl and Wehr, 2008). These results fit well in timeline with the increases of AC firing rates and evoked potentials recorded in our experiment. Therefore, the enhanced cortical neural activity recorded in awake rats may be due to the acute reduction of the cortical inhibition.

3.2. Noise exposure on sound sensitivity

We also monitored the rat’s behavioral response to loud sound using the acoustic startle reflex tests. Interestingly, the acoustical startle amplitude increased after noise exposure at the super-threshold levels. These extraordinary acoustic startle responses suggest noise exposure may increase...
sound loudness reaction—an animal’s behavior which may be related with hyperacusis.

The pathophysiology of hyperacusis, commonly defined as reduced sound tolerance (Vernon, 1987), is still largely unknown. Interestingly, hyperacusis is often reported as a symptom in various neurological diseases, including Williams syndrome (Gothelf et al., 2006), migraine (Woodhouse and Drummond, 1993), and tinnitus (Dauman and Bouscau-Faure, 2005). For example, during migraine attacks, migraine patients often are very sensitive to sound and their uncomfortable level to loud sound drops. Ambrosini et al. found enlarged acoustic evoked cortical potentials in migraine patients and suggests migraine attack can reduce central habituation to the repetitive acoustic stimuli (Ambrosini et al., 2001; Ambrosini et al., 2003).

Hyperacusis is a common symptom reported in people who were often exposed to loud sound, such as in the military service and in the construction industry (Axelsson and Hamernik, 1987). These patients often reported immediate persistent hearing loss, tinnitus, pain and hyperacusis after impulse or blast wave noise. In this study we found that the cortical neurons showed enhanced responses right after noise exposure, suggesting an immediate effect on cortical neural response by noise exposure. As the exaggerated acoustic startle responses happened right after noise exposure, it is possible that the impairments of the central nervous system contribute to the behavioral reaction to sound. Since over reaction (startle, cry, pain) to sudden loud sound is a common phenomenon in human with low sound tolerance (hyperacusis) (Gothelf et al., 2006; Jansen et al., 2009), we think the increased acoustic startle response may be indicative of hyperacusis behavior (Ison et al., 2007; Sun et al., 2009).

Hyperacusis is often reported in patients with tinnitus. Eighty-six percent of the patients whose chief complaint is hyperacusis also reported tinnitus (Anari et al., 1999). It is possible that these two abnormal sound perceptions may be caused by the same mechanism in some patients. Our recent studies showed that high doses of salicylate, a reliable inducer of tinnitus, can also cause a significant enhancement of sound evoked cortical response and acoustic startle response (Sun et al., 2009; Yang et al., 2007). It suggests that the cortical hyperexcitability may play an important role in hyperacusis as well as in tinnitus. Recently, the approach of using electrical stimulation of the AC to treat patients with tinnitus has been tested (De Ridder et al., 2006; De Ridder et al., 2007). DeRidder et al. reported that patients with pure tone type of tinnitus experienced a significant 97% suppression while those who had noise type tinnitus had non-significant suppression. Based on these studies, the phantom sensation of tinnitus may be related with the abnormal cortical activities...
and the electrical stimulation on sensory cortices could be served as an effective treatment on severe unilateral tinnitus in some patients.

In summary, our experiments demonstrated that noise exposure can cause an acute AC hyperactivity and a temporary increase of acoustic startle response. Our results suggest that the hyperacusis behavior caused by noise exposure may be related to the hyperactivity of cortical neurons.

4. Experimental procedures

4.1. Animals

Twenty-five adult male Sprague Dawley SD rats (3–6 months old, 300–500 g, Harlan Laboratories, Indianapolis, IN) were used in the electrophysiological test (n=17) and the behavioral test (n=8). All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the State University of New York at Buffalo and were consistent with the guidelines issued by the National Institutes of Health.

4.2. Hyperacusis behavioral assessment

The acoustic startle amplitude has been used to evaluate hyperacusis behavior (Ison et al., 2007; Sun et al., 2009). During the test, each animal was placed in an acoustically transparent wire mesh cage (7×5.5×18 cm) mounted on a Plexiglass base which was rested on a sensitive piezoelectric transducer (Radio Shack). The output of the piezo transducer was passed through a low-pass filter (1000 Hz, LPF-300, World Precision Instruments, Sarasota, FL) and then sent to an A/D converter on an RP2 Real-time Processor (Tucker-Davis Technologies, TDT). The root mean square (rms) of the response (100 ms window) was measured using custom software. The sound stimuli were presented by a high frequency speaker (Fostex FT28D) located approximately 28 cm above the rat’s head. Sound signals were generated by RP2 Real-time Processor (TDT) controlled by custom software.

The acoustic startle response for the hyperacusis test was evoked by a narrowband noise burst (50 ms duration) centered at 8 or 16 kHz with 1000 Hz bandwidth from 60 to 110 dB SPL (10 dB step). The stimuli were presented 10 times at each intensity in a pseudo-random order. The inter-trial interval was varied from 18 to 22 seconds. The amplitude of the acoustic startle response was measured in a 100 ms window after the onset of the acoustic stimulus.

4.3. Noise exposure and hearing evaluation

Rats were exposed to a narrowband noise centered at 12 kHz (1 kHz bandwidth) at 120 dB SPL for 1 hour in a sound proof room. Sound stimuli were generated by a sound processor (RP2, TDT, Alachua, FL, USA) and presented by a loud speaker (GMI D-49, GMI Sound Corp., Brooklyn, NY) positioned 10 cm in front of the rat’s head to expose both ears. The sound pressure level was calibrated with a sound level meter coupled to a half-inch condenser microphone (Model 824 Audiometer, Larson Davis).

The auditory brainstem response (ABR) was recorded in anesthetized rats before and after noise exposure to monitor their hearing threshold. ABR was tested in a sound proof room with BioSigRP Software (TDT) and TDT hardware (Sun et al., 2009).

4.4. Electrode implantation for the chronic recording

Eight rats were implanted with low impedance customized electrodes made of Teflon coated tungsten wire (0.3 mm diameter, A-M Systems, W.A.) in the IC and the AC for the field potential recordings. During the surgery, rats were anesthetized with ketamine (50 mg/kg) and xylazine (6 mg/kg) and their heads were fixed in a stereotaxic frame (WPI). Then the surface of the parietal bone and part of the frontal bone were exposed. Four small stainless steel screws (Small Parts Inc.), two in the frontal bone and two in the parietal bone, were inserted into the skull. A thin layer of dental cement was covered on the skull and the screws to provide anchoring. A custom threaded rod (1/2" long with ¼" diameter) was attached to the skull with the dental cement, which would later be used as a headpost during subsequent recording sessions. A stainless steel ground electrode was implanted under the dura and fixed on the surface of the parietal bone.

For the AC electrode implantation, a 1–2 mm diameter hole was opened in the skull over the AC (5–6 mm posterior to bregma on the internal side of the suture between the parietal and the temporal bones (Polley et al., 2007)). For the IC electrode implantation, a 1–2 mm diameter hole was exposed in the skull over the IC (1–2 mm posterior to the lambda and 2 mm lateral to the midline). The electrode was mounted on a stereotaxic manipulator and was gently advanced through the opening in the skull using a hydraulic manipulator. A sweep tone was presented as the electrode was advanced into the AC or the IC. The response from the electrode was amplified using TDT System-3 (TDT) hardware and the response was monitored. Once a synchronized AC or IC response was recorded, the electrode was fixed to the skull. Then the wound was sutured around the electrode connector. The animal was allowed to recover for 3–5 days before the recording.

In order to record the multiunit response in the AC, 8 rats were implanted with a high impedance electrode (0.5–6 MΩ, 75 µm diameter, FHC, ME) for the single unit recordings. The procedure of surgery is the same as that of the field potential recording. The detailed method can be found in our previous paper (Yang et al., 2007).

4.5. Acoustic stimuli and recordings

The acoustic signals were generated by SigGen (TDT) and the sound was presented by a high frequency speaker to the contralateral ear of the recorded the IC and AC (Fostex FT28D, Tokyo, Japan). Tone-bursts (50 ms duration, 1 ms rise/fall time) were used to elicit responses; sound intensity was varied from 10 to 90 dB SPL (10 dB step). During the recording, the rats were restrained in the testing apparatus and the head was fixed by the headpost (Yang et al., 2007). The output of the AC or IC response was connected to a 16 channel preamplifier (RA16PA, TDT) through a flexible low noise cable.
The output of the preamplifier was delivered to a digital signal processing module (RXS-2, Pentusa Base Station, TDT) connected to a computer. Peri-stimulus time histograms (PSTH) were constructed from spike discharges (100–3000 Hz) using Brainware software (TDT). The average firing rate of the AC neurons was calculated using a 50 m² window when the sound stimulus was presented. The acoustic intensity was calibrated using a sound level meter (824, Larson Davis, Depew, NY) with ½” condenser microphone (Larson Davis). All the physiological tests were performed in a sound proof room.

4.6. Statistic data analysis

Graphs and statistic analyses were generated using GraphPad Prism (Version 5, GraphPad Software, San Diego, CA, USA). Results are presented as mean±standard error of the mean.

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