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BLAST-INDUCED TINNITUS AND HYPERACTIVITY IN THE AUDITORY CORTEX OF RATS

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Abstract—Blast exposure can cause tinnitus and hearing impairment by damaging the auditory periphery and direct impact to the brain, which trigger neural plasticity in both auditory and non-auditory centers. However, the underlying neurophysiological mechanisms of blast-induced tinnitus are still unknown. In this study, we induced tinnitus in rats using blast exposure and investigated changes in spontaneous firing and bursting activity in the auditory cortex (AC) at one day, one month, and three months after blast exposure. Our results showed that spontaneous activity in the tinnitus-positive group began changing at one month after blast exposure, and manifested as robust hyperactivity at all frequency regions at three months after exposure. We also observed an increased bursting rate in the low-frequency region at one month after blast exposure and in all frequency regions at three months after exposure. Taken together, spontaneous firing and bursting activity in the AC played an important role in blast-induced chronic tinnitus as opposed to acute tinnitus, thus favoring a bottom-up mechanism.

Key words: blast, tinnitus, traumatic brain injury (TBI), auditory cortex, spontaneous activity, bursting, rat.

INTRODUCTION

Tinnitus, the perception of sound without an external source, is a complex condition occurring in an estimated 10–15% of the American population (Henry et al., 2005). While there are many causes of tinnitus, high-pressure blast shock waves are often the ones that contribute to both tinnitus and hearing loss in military service members (Geckle and Lee, 2004). In addition to the audiological impact, blast exposure can lead to traumatic brain injury, with one study observing that over 53% of patients recovering from traumatic brain injury developed tinnitus symptoms (Jury and Flynn, 2001). Previous studies have indicated that the occurrence of both injury and compensatory plastic changes in central auditory structures after blast exposure may trigger tinnitus development (Cave et al., 2007; Mao et al., 2012). Nevertheless, there is still a lack of effective treatments for blast-induced tinnitus, largely because of a limited understanding of its underlying neuropathophysiology.

Over the last two decades, numerous studies have suggested that noise-induced tinnitus is correlated with changes in spontaneous activity, neurosynchrony and maladaptive plasticity in the auditory system following acoustic trauma (Bauer, 2004; Eggermont and Roberts, 2004; Eggermont, 2007; Roberts et al., 2010; Eggermont and Tass, 2015; Wu et al., 2016). Increased spontaneous firing rates (SFRs) of neurons in the central auditory system are one potential neural substrate of tinnitus (Eggermont and Roberts, 2004; Wu et al., 2016). Specifically, an increase in SFRs has been found in the dorsal cochlear nucleus (DCN) (Zhang and Kaltenbach, 1998; Wu et al., 2016), ventral cochlear nucleus (Vogler et al., 2011), inferior colliculus (IC) (Bauer et al., 2008) and auditory cortex (AC) (Norena and Eggermont, 2003; Llano et al., 2012). In addition, spontaneous bursting in the auditory system has been suggested to subserve tinnitus (Roberts et al., 2010; Wu et al., 2016). For example, it has been reported that chronic increases in bursting activity occurs in the DCN (Finlayson and Kaltenbach, 2009; Wu et al., 2016) and IC (Bauer et al., 2008) following noise trauma. However, it is not clear whether noise trauma induces a lasting increase of bursting activity in the AC (Norena and Eggermont, 2003). Furthermore, it remains to be determined whether blast-induced tinnitus shares the same neural mechanisms as noise-induced tinnitus.

Although tinnitus appears to result from aberrant neural activity in the auditory system (Jastreboff, 1990; Eggermont and Roberts, 2004), it is unclear which levels of the auditory system are most closely involved in tinnitus generation. Recently, our lab showed that increased spontaneous activity in the DCN and IC of rats with behavioral evidence of tinnitus occurred immediately following blast exposure and lasted for up to one month in the DCN and three months in the IC (Luo et al., 2014a, b). Since noise-induced tinnitus has been proposed to be related to increased SFRs in the AC at both acute (Kimura and Eggermont, 1999; Norena et al., 2003) and

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Abbreviations: AC, auditory cortex; CF, characteristic frequency; DCN, dorsal cochlear nucleus; FTC, frequency tuning curve; IC, inferior colliculus; SFRs, spontaneous firing rates.

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chronic stages (Eggermont, 2000; Seki and Eggermont, 2003), one wonders how neural activity changes in the AC manifest during blast-induced acute and chronic tinnitus. Compared to activity change patterns in the DCN and IC, the information on AC activity will also help delineate whether blast-induced spontaneous firing and bursting activity takes a bottom-up or top-down approach during tinnitus development.

In the current study, we investigated changes in SFRs and bursting activity in the AC of rats with blast-induced tinnitus and characterized the time course of the induced neural activity changes. Specifically, we sought to establish how hyperactivity emerged over a three-month period following blast exposure and to determine whether the magnitude of the induced hyperactivity remained constant or shifted over time. Our results demonstrated robust hyperactivity in all frequency regions of the AC in tinnitus-positive rats at three months post-blast. Combined with our previous studies, this indicates that initial blast-induced tinnitus may be mediated more at the brainstem level, whereas blast-induced chronic tinnitus may reside more within the AC.

EXPERIMENTAL PROCEDURES

Animal subjects

A total of thirty-nine adult male Sprague–Dawley rats (70–80 days old at the beginning of experiments) were used for this study. It should be noted here that data collected from the DCN and IC of the same animals have been published in two separate papers, and detailed experimental procedures have been provided (Luo et al., 2014a,b). Briefly, the rats were divided into three post-blast survival groups: one day, one month and three months post-blast exposure. Each group contained nine blasted rats and four age-matched controls. Experimental rats were exposed to a single 22-psi (equivalent to 197.5 dB peak SPL) blast with the left ear open and the right ear occluded with an earplug plus mineral oil. All experimental procedures were carried out by following the guidelines of the institutional Animal Care and Use Committee at Wayne State University.

Electrophysiology recording

Electrophysiological recordings in the AC were performed in each rat to examine blast-induced neural activity changes. Briefly, each animal was anesthetized with a mixture of air (1 l/min) and isoflurane (5% v/v). Before inserting the electrode, a craniotomy was performed to provide access to the right AC. To reach the AC, anatomical landmarks of the right side skull were used and the dura mater above the AC was removed. Using a micromanipulator (Kopf Model 1460-61), a custom-made 16-channel microwire electrode array was inserted into the AC. Each electrode array consisted of 16 polyimide-insulated platinum/iridium microwires. They were arranged in two rows with eight wires in each row. The parameters of the array were: diameter = 50 μm, electrode spacing = 400 μm, row spacing = 400 μm, and impedance = 20–50 kΩ. The electrode array was inserted into the AC at 0.8–0.9 mm from its surface, which corresponds to layers 4–5 of the cortex (Hughes et al., 2010). Prior to insertion, the array was dipped in 3% DiI solution (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindo carbocyanine perchlorate, Invitrogen) prepared with N,N-dimethylformamide (DMF) to label the electrode insertion tracks. After placement of the array, the exposed cortex was covered with 2% agar to prevent desiccation. The output from the headstage channels was connected to a real-time auditory electrophysiological station (RZ2, TDT). The station's settings were: 25-kHz sampling rate, 100- to 3000-Hz bandpass filter, and threshold at 1.5 times the standard deviation. The frequency tuning curve (FTC) properties of all recording sites were determined in response to tone pips with different frequency–intensity combinations. Spontaneous single- and multi-unit spikes were recorded twice; once 5 min prior to and another 5 min after FTC construction. Each spontaneous recording period lasted 5 min. Spontaneous activity data collected after FTC construction were used for data analyses, since they were more stable than those collected before FTC construction. At the end of each recording, the rat was euthanized and its brain removed and processed histologically. The electrode tracks were later examined with the aid of a Nikon fluorescent microscope (Eclipse E400).

Data analysis

Characteristic frequency (CF) of each recording site was determined by defining the frequency of a stimulus tone at the lowest intensity level needed to evoke neuronal activity. CFs were determined using automated algorithms and, as expected, each channel produced a clearly defined CF. To avoid misrepresentation of CF-tagged spike activity caused by variable electrode penetrations in the AC across different rats, data from recording sites of three adjacent frequency bands based on their CFs were pooled into three separate groups. Specifically, sites with CFs at 2–4 kHz were grouped to represent a low-frequency band, those with CFs at 4–16 kHz were grouped to represent an intermediate frequency band, and those at 16–42 kHz were grouped to represent a high-frequency band.

Bursting activity was detected using the Poisson-surprise method (Legendy and Salcman, 1985) with Neuroexplorer software (Nex Technological). In this method, it is assumed that a spike train has a Poisson distribution, and the negative binary logarithm of the probability of the occurrence of a given number of spikes in a designated time interval is considered. When the Poisson-surprise value increases, spikes in a burst are added from at least three spikes. Only bursts with a surprise value $\geqslant 4$, indicating that they occurred at least 16 (2^4) times as frequently as in a Poisson spike train with the same firing rate, were considered in the analysis.

Statistically, the status of tinnitus and recovery time points after blast exposure were used as two factors, and the three frequency regions were individually tested. The distribution of the data values from each frequency band passed normality (Kolmogorov–Smirnov). Thus ANOVA and multivariate analysis were used to