Blast-Induced Tinnitus and Hyperactivity in the Auditory Cortex of Rats

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Abbreviations:

ABR	auditory brainstem response
AC	auditory cortex
CF	characteristic frequency
DCN	dorsal cochlear nucleus
FTC	frequency tuning curve
IC	inferior colliculus
SFRs	spontaneous firing rates

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 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 auditory cortex played an important role in blast-induced chronic tinnitus as opposed to acute tinnitus, thus favoring a bottom-up mechanism.

Keywords: Blast, Tinnitus, Traumatic brain injury (TBI), Auditory cortex, Spontaneous activity, Bursting, Rat

1 **Introduction**

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

13 Over the last two decades, numerous studies have suggested that noise-induced tinnitus is
14 correlated with changes in spontaneous activity, neurosynchrony and maladaptive plasticity in
15 the auditory system following acoustic trauma (Bauer, 2004, Eggermont and Roberts, 2004,
16 Eggermont, 2007, Roberts et al., 2010, Eggermont and Tass, 2015, Wu et al., 2016). Increased
17 spontaneous firing rates (SFRs) of neurons in the central auditory system are one potential neural
18 substrate of tinnitus (Eggermont and Roberts, 2004, Wu et al., 2016). Specifically, an increase in
19 SFRs has been found in the dorsal cochlear nucleus (DCN) (Zhang and Kaltenbach, 1998, Wu et
20 al., 2016), ventral cochlear nucleus (Vogler et al., 2011), inferior colliculus (IC) (Bauer et al.,
21 2008) and auditory cortex (AC) (Norena and Eggermont, 2003, Llano et al., 2012). In addition,
22 spontaneous bursting in the auditory system has been suggested to subserve tinnitus (Roberts et
23 al., 2010, Wu et al., 2016). For example, it has been reported that chronic increases in bursting

activity occurs in the auditory nerve (Liberman and Kiang, 1978), DCN (Finlayson and Kaltenbach, 2009, Wu et al., 2016) and IC (Chen and Jastreboff, 1995) 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., 2014ab). 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 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., 2014ab). 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 k Ω . 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'-tetramethylindocarbocyanine 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, 100-3000 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 minutes. 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 a middle 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 ≥ 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 was used to determine significant changes. Post-hoc analyses were performed using Bonferroni tests. Differences were considered to reach statistical significance when $p < 0.05$.

Results

As shown in our recent study (see Figures 1-2 in (Luo et al., 2014b), there were nine tinnitus positive rats at one day following blast exposure, six tinnitus positive and three tinnitus negative rats at one month after blast, and four tinnitus positive and five tinnitus negative rats at three months after blast. Significant hearing threshold elevations were found in the exposed ears of rats at one day after blast, while rats exhibited threshold recovery at one and three months after blast exposure. In spite of threshold recovery, however, P1-N1 amplitudes were significantly reduced in both tinnitus positive and negative rats compared to control rats. This indicated that significant hearing impairment remained at certain frequencies.

Changes in spontaneous firing rate

At one day after blast exposure, there were no significant SFR differences in any frequency regions between tinnitus positive rats and control rats (Figure 1). One month after exposure, moderately decreased SFRs were found in the 16-42 kHz region in both tinnitus positive and tinnitus negative rats compared to the controls ($F_{(2, 49)} = 4.347, p < 0.05$), but no significant difference was reached between tinnitus positive and control animals in between-group tests (one-way ANOVA with Bonferroni post-hoc tests). At three months after blast exposure, SFR changes were quite different from those found at one day and one month after exposure. The SFR in tinnitus positive rats increased in all frequency regions compared to tinnitus negative or control rats. These increases reached statistical significance at 2-4 kHz ($F_{(2, 51)} = 7.994, p < 0.01$), 4-16 kHz ($F_{(2, 150)} = 19.322, p < 0.01$), and 16-42 kHz ($F_{(2, 51)} = 9.384, p < 0.01$). However, no significant difference was found between tinnitus negative and control rats (one-way ANOVA with Bonferroni post-hoc tests). We also compared the SFRs of each

frequency region to assess which brain region in the AC was more sensitive to blast exposure. Our results showed significant changes at 2-4 kHz ($F_{(3, 143)} = 4.626, p < 0.01$), 4-16 kHz ($F_{(3, 143)} = 4.506, p < 0.01$), and 16-42 kHz ($F_{(3, 143)} = 7.590, p < 0.01$), but there were no significant differences among the status of tinnitus and recovery time points (two-way ANOVA with Bonferroni post-hoc tests) (Figure 2).

Changes in bursting rate

As seen in Figure 3, there were no bursting rate changes in any frequency regions between tinnitus positive and control rats at one day after blast exposure. At one month after blast, bursting rates increased at the 2-4 kHz and 4-16 kHz regions in tinnitus positive rats. The increase was statistically significant only in the 2-4 kHz region ($F_{(2, 45)} = 3.285, p < 0.05$), but there was no significant difference between tinnitus positive and control rats in between-group tests (one-way ANOVA with Bonferroni post-hoc tests). Three months after blast exposure, bursting rates in tinnitus positive rats were significantly increased across all frequency regions: 2-4 kHz ($F_{(2, 51)} = 4.160, p < 0.05$); 4-16 kHz ($F_{(2, 155)} = 15.471, p < 0.01$); 16-42 kHz ($F_{(2, 51)} = 6.173, p < 0.01$) (one-way ANOVA with Bonferroni tests). The significant differences were found for the within-group tests, however, and did not include the tinnitus positive and tinnitus negative rats at the 2-4 kHz region or the tinnitus negative and control rats in any frequency regions. Similar to the analysis for SFR data, rats' tinnitus status and time points after blast exposure were used as two factors. No statistically significant differences in bursting activity change were found between frequency regions ($p > 0.05$) (Figure 4).

Discussion

The results described here extended upon our previous findings that blast-induced tinnitus, observed over a three month period, is accompanied by immediate hyperactivity that later shifted to lower SFRs in the DCN and by persistent hyperactivity in the IC (Luo et al., 2014ab). In the current study, we found hyperactivity in the AC of tinnitus positive rats that did not appear immediately after blast exposure, but later became prominent in all frequency regions at three months after blast exposure. This delayed appearance of blast-induced hyperactivity in the AC suggests that it became intrinsic, independent of afferent inputs from the cochlea, and thus a possible basis for chronic tinnitus.

Blast-induced hyperactivity in the AC

The current animal study for the first time elucidated neurophysiological mechanisms underlying blast-induced tinnitus in the AC. Different animal models have demonstrated that noise trauma can cause an increase in SFRs and bursting activity in the AC (Norena and Eggermont, 2003, 2005, 2006, Roberts et al., 2010). Such hyperactivity can occur immediately after noise trauma (Norena and Eggermont, 2003) and last for several weeks (Norena and Eggermont, 2006). Interestingly, we did not find increased SFR at one day and one month after blast exposure. Instead, we found that SFRs increased at three months after blast exposure, but only in tinnitus positive animals. Our previous studies showed that tinnitus developed one day after blast exposure and was accompanied by significant elevation of SFRs in the DCN and IC. These results suggest that acute tinnitus may result from hyperactivity in the lower auditory brainstem, whereas chronic tinnitus may be related to higher-level auditory structures.

Mechanistically, tinnitus may result from spontaneous activity changes in the auditory system (Eggermont and Roberts, 2004), which often co-occur with a shifted balance between excitatory and inhibitory inputs to tinnitus-generating neurons or neuronal populations. The decrease in inhibition may result from weakened GABAergic neurotransmission, such as that observed in the IC following acoustic trauma (Richardson et al., 2012). The GABAergic neurotransmission could have mediated the increased SFRs in the AC. Our recent finding that SFRs in the IC gradually increased after blast exposure suggests a progressive decrease in inhibition (Luo et al., 2014a). Our findings are in line with the notion that tinnitus-related hyperactivity at the cortical level may result from pathologically propagated gain change that is originally developed in the brainstem. Abnormal spontaneous activity in the AC appears to result from over-amplified spontaneous firing along the auditory pathway, which starts from the cochlear nucleus (Schaette and Kempster, 2012).

Blast-induced bursting changes in the AC

Bursting firing is known to enhance the reliability of synaptic transmission and neuronal selectivity, thus triggering synaptic plasticity (Swadlow and Gusev, 2001). Bursting activity also enriches functional values of cortical activity, especially regarding serial synchrony of spontaneous firings in the cortex (Roberts et al., 2010). Previously, it has been reported that bursting activity in the AC varies over time following noise trauma. Specifically, noise trauma may induce immediate increase of burst firing, which later returns to a pre-exposure level (Norena and Eggermont, 2003). However, the current study demonstrated significantly increased bursting rate in the AC of tinnitus positive rats only at one and three months after blast exposure. This increase encompassed all frequency regions at three months after blast exposure. The

mechanisms underlying the changes in bursting activity following trauma remain unclear. It has previously been suggested that bursting activity may reflect the balance between excitation and inhibition (Krishnan and Plack, 2009). Blast exposure, through its severe mechanical impact on the cochlea and brain, may have changed bursting activity through a modification of this balance. To clearly answer this question, further investigations are needed to delineate the underlying differences between noise- and blast-induced changes in bursting activity at a single cell level.

Neural mechanisms of blast-induced acute and chronic tinnitus

Acoustic trauma-induced anatomical, neurochemical and neurophysiological changes in both the peripheral and central auditory system have been well studied and documented (Browne et al., 2012, Mulders and Robertson, 2013, Shore et al., 2016). Following acoustic trauma, the induced cochlear damage often manifests as immediate loss of afferent inputs to portions of the cochlear nuclei and higher-level structures of the auditory system. Such a rapid loss of afferent inputs often results in diminished inhibition (Bilak et al., 1997, Mulders et al., 2011), which in turn may cause rapid unmasking effects on complex neural circuits in the impacted structures (Chambers et al., 2016). At the same time, homeostasis-related neuroplasticity occurs, which can be in the forms of sprouting of new axons, development of new synapses, and modifications of the neurochemical environment. These above changes may result in immediate and chronic neurophysiological changes (Kaltenbach, 2011). Recently, we demonstrated immediate hyperactivity one day after blast and sustained hyperactivity in the DCN and IC through one month after blast exposure, although this shifted to lower SFRs in the DCN and persistent hyperactivity in the IC (Luo et al., 2014ab). Together with these findings in the DCN and IC, the present observations lend support to the hypothesis that, immediately after blast exposure, the

increased spontaneous activity in the DCN and the IC may be due to hyperexcitability of neurons. However, over a period of three months following blast exposure, this early stage of neuroplasticity transitions to one in which elevated firing may become intrinsic to the neurons in the IC or AC. Such results may support a hypothesis that acute tinnitus may result more from aberrant activity in the lower-level auditory structures, while chronic tinnitus may result more from abnormal neural activity in the higher-level auditory structures. In spite of this, the results from the current study do not rule out the possibility that the AC is still involved in acute tinnitus. Independent or combined measures of neurosynchrony and/or neurocoherence within and between auditory or even non-auditory centers are needed to address the important role that neural interaction and connectivity play in blast-induced neural propagation along the auditory pathways (Zhang et al., 2015).

Conclusions

Our results demonstrate that blast-induced tinnitus may not involve a uniform manifestation of hyperactivity in the auditory cortex. Instead, moderately decreased spontaneous firing occurred in the AC at one month after blast, whereas robust hyperactivity occurred across all the frequency regions at three months after blast. These changes were accompanied by increased bursting activity. The current findings together with those from our recent studies in the DCN and IC suggest that blast-induced acute tinnitus may be related to increased neural activity in the lower-level auditory centers while chronic tinnitus may be attributed to abnormal neural activity in the higher-level auditory centers. Further understanding of the neural mechanisms underlying blast-induced tinnitus will help develop targeted preventative and therapeutic strategies.

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Figure Legends

Figure 1. SFRs recorded in three frequency regions (2-4 kHz, 4-16 kHz and 16-42 kHz) in the AC of tinnitus positive, tinnitus negative and age-matched control rats at one day, one

month, and three months after blast exposure. For the tinnitus positive group, note the significant decrease in 16-42 kHz SFR at one month after exposure, and the significant increase across all frequency regions at three months after exposure. Error bars represent standard error of the mean. * $p < 0.05$.

Figure 2. Plot showing the temporal profile of the effects of blast-induced tinnitus and hearing impairment on SFRs for the control, tinnitus negative and tinnitus positive groups. Error bars represent standard error of the mean. * $p < 0.05$.

Figure 3. Bursting activity measured in three frequency regions (2-4 kHz, 4-16 kHz and 16-42 kHz) in the AC of tinnitus positive, tinnitus negative, and age-matched control rats at one day, one month, and three months after blast exposure. Note the significant elevation in bursting rates in the 2-4 kHz region at one month after blast and across all frequency regions at three months after blast. Error bars represent standard error of the mean. * $p < 0.05$.

Figure 4. Plot showing the temporal profile of the effects of blast-induced tinnitus and hearing impairment on bursting activity for the control, tinnitus negative and tinnitus positive groups. No statistically significant differences in bursting activity change were found between frequency regions ($p > 0.05$). Error bars represent standard error of the mean.

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