Neuroscience 304 (2015) 368–377

NOISE-INDUCED DAMAGE TO RIBBON SYNAPSES WITHOUT PERMANENT THRESHOLD SHIFTS IN NEONATAL MICE

L. SHI,[a†] X. GUO,[a,b†] P. SHEN,[a†] L. LIU,[a] S. TAO,[a] X. LI,[a] Q. SONG,[c] Z. YU,[d] S. YIN[c*] AND J. WANG[a,d*]

[a] *Department of Physiology, Medical College of Southeast University, 87 Dingjiaoqiao Road, Nanjing 210009, China*

[b] *Children's Medical Center, The Second Affiliated Hospital of Nanjing Medical University, 262 Zhongshan Road North, Nanjing 210003, China*

[c] *Department of Otolaryngology, 6th Affiliated Hospital, Jiaotong University, 600 Yishan Road, Shanghai 200233, China*

[d] *School of Human Communication Disorders, Dalhousie University, 1256 Barrington Street, Halifax, NS B3J1Y6, Canada*

Abstract—Recently, ribbon synapses to the hair cells (HCs) in the cochlea have become a novel site of interest in the investigation of noise-induced cochlear lesions in adult rodents (Kujawa and Liberman, 2009; Lin et al., 2011; Liu et al., 2012; Shi et al., 2013). Permanent noise-induced damage to this type of synapse can result in subsequent degeneration of spiral ganglion neurons (SGNs) in the absence of permanent changes to hearing sensitivity. To verify whether noise exposure during an early developmental period produces a similar impact on ribbon synapses, the present study examined the damaging effects of noise exposure in neonatal Kunming mice. The animals received exposure to broadband noise at 105-decibel (dB) sound pressure level (SPL) for 2 h on either postnatal day 10 (P10d) or postnatal day 14 (P14d), and then hearing function (based on the auditory brainstem response (ABR)) and cochlear morphology were evaluated during either postnatal weeks 3–4 (P4w) or postnatal weeks 7–8 (P8w). There were no significant differences in the hearing threshold between noise-exposed and control animals, which suggests that noise did not cause permanent loss of hearing sensitivity. However, noise exposure did produce a significant loss of ribbon synapses, particularly in P14d mice, which continued to increase from P4w to P8w. Additionally, a corresponding reduction in the amplitude of compound action potential (CAP) was observed in the noise-exposed groups at P4w and P8w, and the CAP latency was elongated, indicating a change in synaptic function.

Key words: noise, permanent threshold shift, ribbon synapse, cochlea, neonatal mice.

INTRODUCTION

Noise exposure is the most common cause of acquired sensorineural hearing loss (SNHL) in modern society (NIDCD, 1995; Stucken and Hong, 2014), but the mechanisms underlying this type of hearing loss remain unclear. A majority of studies have focused on mechanical damage and the deleterious effects of noise exposure on outer hair cells (OHCs) as the primary factors involved in this process (Ivory et al., 2014; Stucken and Hong, 2014; Furness, 2015). While the damaging effects of noise on ribbon synapses between spiral ganglion neurons (SGNs) and cochlear hair cells (HCs), particularly inner hair cells (IHCs), have also been assessed by previous studies, (Puel et al., 1997; Pujol and Puel, 1999), this type of damage was largely considered to be repairable and, therefore, not important. More recently, however, quantitative studies have demonstrated the development of permanent noise-induced damage in ribbon synapses, which suggests that the repair of these synapses following such damage is incomplete (Kujawa and Liberman, 2009; Lin et al., 2011; Liu et al., 2012; Shi et al., 2013).

For example, in adult rodents (both mice and guinea pigs), brief exposure to relatively low-level noise that does not cause a permanent threshold shift (PTS) in hearing can produce massive damage to the ribbon synapses (Kujawa and Liberman, 2009; Lin et al., 2011; Liu et al., 2012; Shi et al., 2013). Without a PTS, this type of damage would likely go unnoticed by human subjects and would almost certainly be missed during routine audiology evaluations that focus primarily on hearing threshold levels. Therefore, this kind of acoustic trauma might be silent or subclinical. Even though noise-induced damage to the ribbon synapses may be partially repairable, permanent damage to these synapses can result in the

*Corresponding authors at: School of Human Communication Disorders, Dalhousie University, 1256 Barrington Street, Halifax, NS B3J1Y6, Canada. Tel: +1-9024945149; fax: +1-902 4945151 (J.Wang) and Dept. of Otolaryngology, 64h Affiliated Hospital, Jiaotong Unversity, Shanghai, 200233, China. Tel: 8621-64834143 (S. Yin).

E-mail addresses: shilj_seu@163.com (L. Shi), guoxiaojing0818@126.com (X. Guo), spei1020@sina.cn (P. Shen), liulj321@163.com (L. Liu), 695377378@qq.com (S. Tao), lixiaowei6138@126.com (X. Li), 13816506336@126.com (Q. Song), zyu@dal.ca (Z. Yu), yinshankai@china.com (S. Yin), Jian.Wang@dal.ca (J. Wang).

† These authors contributed equally.

Abbreviations: ANOVA, analysis of variance; CAP, compound action potential; CaV1.3, voltage-sensitive calcium channels; CtBP2, C-terminal-binding protein 2; dB, decibel; HCs, hair cells; IHCs, inner hair cells; OHCs, outer hair cells; PBS, phosphate-buffered saline; PSD, postsynaptic density; PTS, permanent threshold shift; P4w, postnatal week 4; P8w, postnatal week 8; P10d, postnatal day 10; P14d, postnatal day 14; SEM, standard errors of the mean; SGNs, spiral ganglion neurons.

http://dx.doi.org/10.1016/j.neuroscience.2015.07.066

degenerative death of SGNs that are disconnected from the IHCs (Kujawa and Liberman, 2009; Lin et al., 2011). Furthermore, although noise-induced damage can occur in the absence of hearing loss (as defined by threshold elevations), there may still be functional deficits in cochlear coding. More specifically, this type of massive damage will likely result in a significant reduction in cochlear output to the auditory cortex that can be identified by reductions in the amplitude of the compound action potential (CAP) and, ultimately, impairments in the coding of sound intensity.

The synaptic ribbon, which is a characteristic structure of ribbon synapses located mainly in the inner ear and on the retina (Nouvian et al., 2006), is responsible for the fast release of neurotransmitters by HCs (Fuchs et al., 2003; Fuchs, 2005; Sterling and Matthews, 2005; Nouvian et al., 2006; Moser et al., 2006a,b). Therefore, this structure likely plays a critical role during temporal resolution in the cochlea (Nouvian et al., 2006; Moser et al., 2006a,b; Schmitz, 2009). The importance of ribbon synapses in the cochlea has been demonstrated in animal studies involving a mutation in Bassoon, a critical ribbon protein (Buran et al., 2010). In these mutant cochlea, the synapses between SGNs and IHCs are of the conventional type without ribbons, and although the mutated animals show no differences from normal controls in single-unit thresholds, their temporal coding ability is largely reduced (Buran et al., 2010; Jing et al., 2013). Additionally, a recent study from our group revealed that the damage and repair of ribbon synapses resulting from noise exposure is likely accompanied by deterioration in temporal processing (Shi et al., 2013). The clinical implications of noise-induced damage are important. Because it is more likely that an individual will be exposed to noise that does not result in a change in PTS than to a stronger stimulus in an industrial setting, the deterioration in temporal processing that occurs with aging may be due, at least in part, to the ''silent'' noise-induced damage of ribbon synapses. Moreover, these effects may go unnoticed and accumulate with age.

To our knowledge, all previous investigations of noise-induced damage in cochlear ribbon synapses have been conducted in adult animals. However, accumulating evidence suggests that auditory sensory organs appear to be more sensitive to a variety of ototoxic factors, including noise, during early development (Hall, 2000; Surenthiran et al., 2003; Li and Steyger, 2009; Reeves et al., 2010). The postnatal period in rodents has long been identified as one that is sensitive to acoustic trauma (Lenoir and Pujol, 1980; Saunders and Chen, 1982), but early studies focused primarily on noise damage to OHCs. To date, no studies have investigated the effects of noise on ribbon synapses in the neonatal mammalian cochleae.

Because exposure to noise during early development is a highly probable occurrence, particularly for preterm infants exposed to life-support systems (Surenthiran et al., 2003; Lahav, 2014; Laubach et al., 2014; Park et al., 2014), a better understanding of the effects of noise-induced damage on ribbon synapses during early development is important for the formulation of noise safety guidelines. Thus, the present study examined the effects of noise-induced silent damage on ribbon synapses in neonatal mice during the early onset of hearing. The present results demonstrated that noise exposure shortly after birth resulted in similar but less marked damage to the ribbon synapses than has been observed in previous studies conducted in adult mice (Kujawa and Liberman, 2009; Lin et al., 2011; Liu et al., 2012; Shi et al., 2013). However, in contrast to adult animals, the loss of ribbon synapses progressed continuously in the young mice for more than 1 month following exposure to noise.

EXPERIMENTAL PROCEDURES

Subjects and experimental outline

Pregnant Kunming mice were obtained from Qinglongshan Animal Farm (Nanjing, Jiangsu, China), which is a qualified provider of laboratory animals, and 36 neonatal mice were recruited from four litters for the present study. The neonatal mice were divided randomly into three groups of equal size (n = 12) with an equal number of males and females. Experimental Group one was exposed to noise on postnatal day 10 (P10d), Experimental Group two was exposed to noise on postnatal day 14 (P14d), and the control group was not exposed to noise. Within each group, the animals were divided into two subgroups (n = 6 each) based on the performance of the end tests: either postnatal week 4 (P4w) or postnatal week 8 (P8w). The six subgroups were labeled such that the timing of both the noise exposure and end tests is indicated, as follows: Ctrl4w, Ctrl8w, Exp10d4w, Exp10d8w, Exp14d4w, and Exp14d8w. For example, Exp14d4w indicates that the mice in this group were exposed to noise on P14d and examined at 4 weeks of age.

The animals in the experimental groups were exposed to broadband noise at a 105-decibel (dB) sound pressure level (SPL) for 2 h on either P10d or P14d, whereas the animals in the control group underwent sham exposure (environmental change) on P14d. All mice were returned to their mothers after the exposure session. The end tests included auditory brainstem response (ABR) and CAP assessments for functional evaluation and morphological examination of the ribbon synapses. All animal procedures were approved by the University Committee for Laboratory Animals of Southeast University, China (Permit number: SYXK 2011-0009).

Noise exposure

The experimental subjects were exposed to the broadband noise while awake and unrestrained in a cage. The floor of the cage was 60 cm below the horns of one low-frequency woofer and one high-frequency tweeter, and electrical Gaussian noise was delivered through the two loudspeakers after power amplification. The acoustic spectrum of the sound was distributed mainly below 20 kHz, as described previously (Liu et al., 2012), and the frequency range for sound density 10 dB below the peak was between 3 and 14 kHz. The noise level was monitored using a 0.25-inch microphone linked to a sound level meter (microphone: 2520, sound level meter: 824, Larson Davis; Depew, NY, USA).