The Functional and Morphological Changes by Noise without Causing Hearing Loss: A Comparison between Mice and Guinea Pigs

by

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ABSTRACT

Noise exposure is one of the most common causes for acquired sensorineural hearing loss (SNHL). Recent studies have demonstrated that low-level noise exposure can kill spiral ganglion neurons (SGNs) without causing permanent hearing threshold shift. The present study further investigated the dynamic changes of ribbons in the cochlea of guinea pigs and the impact of the noise-induced synaptic damage and the loss of SGNs on the temporal processing of the cochlea in both mice and guinea pigs. Unlike what was reported in mice, the initial loss of ribbon in guinea pigs (>60% at high frequency region) largely recovered within one month after the noise, which was consistent with the functional recovery in auditory sensitivity and cochlear response amplitude. However, temporal processing in guinea pigs and mice remained deteriorated long after the hearing threshold was recovered.

LIST OF ABBREVIATIONS USED

SNHL	Sensorineural Hearing Loss
NIHL	Noise-induced Hearing Loss
SGNs	Spiral Ganglion neurons
GP	Guinea pig
IHCs	Inner Hair Cells
OHCs	Outer Hair Cells
dB	Decibel
SPL	Sound Pressure Level
CtBP1	Transcriptional Co-repressor C-terminal Binding Protein 1
CtBP2	Transcriptional Nuclear Co-repressor C-terminal Binding Protein 2
Ca+2	Calcium
AMPA receptor	α-Amino-3-hydroxy-5-Methyl-4-isoxazolepropionic Acid
NMDA	N-Methyl-D-Aspartate receptor
ABR	Auditory evoked Brainstem Response
CAP	Compound Action Potential
pe SPL	Peak Equivalent Sound Pressure Level
I/O functions	Input/output Function
wk	week
d	day
i.p	Intraperitoneal
mm	Millimeter
kHz	Kilohertz
ms	Millisecond
cm	Centimeter
ISI	Inter stimulus interval
HSD	Honestly Significant Difference

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CHAPTER 1 INTRODUCTION

1.1 Introduction

Sensorineural hearing loss (SNHL) is considered as one of the most common neurological disorders (NIDCD, 1995). The most common factors that are known to cause SNHL are excessive noise, exposure to ototoxic drugs, aging, and congenital and genetic defects. Noise is one of the major health concerns in modern life, especially because noise exposure appears to be inevitable. Noise can cause SNHL on its own or exacerbate conditions of SNHL in combination with other factors. Many studies have been conducted on the effect of excessive noise on hearing, but the pathophysiologic mechanisms underlying the noise induced hearing loss are not fully understood.

Currently, the impact of noise on hearing is evaluated mainly through measures of change in auditory sensitivity or threshold after noise exposures. Based on these measures, safety standards for noise exposure are established. However, the threshold-based practice has been challenged by recent studies showing that noise exposure at low levels can kill spiral ganglion neurons (SGNs) silently (without causing a permanent hearing threshold shift). Such a noise-induced SGN death has been verified at least in two species of experimental animals, with a massive loss of SGN in mice and a smaller but still significant loss in guinea pigs (Santi et al., 2008; Kujawa & Liberman, 2009; Lin et al., 2011).

The noise induced SGN death is found to be initiated from damage to ribbon synapses, which appear to be vulnerable to noise (Kujawa & Liberman, 2009). If the damaged synapses remain unrepaired, SGNs will lose the trophic support from hair cells and supporting cells (Kujawa & Liberman, 2009). Although synaptogenesis and nerve terminal regeneration were reported to follow noise exposure (Puel et al., 1998; Pujol & Puel, 1999), the results from these studies were criticized as not being quantitative and not tracking over a sufficiently long term (Kujawa & Liberman, 2009).

The study in mice has shown that after noise exposure there was a quick and massive loss of ribbons, which was possibly accompanied with initial loss of the unmylinated postsynaptic afferent terminals; and a limited repair of ribbon (~10%) (Kujawa & Liberman, 2009). The damaged synapses appeared largely not be repaired because a massive SGN loss was identified 2 years later, which was roughly corresponds to the percentage of the ribbon loss that remained after the initial recovery. This result also suggests that the death of SGN is due to the loss of trophic support from hair cells and supporting cells (Kujawa & Liberman, 2009). In the guinea pig study more recently, the damage to ribbon synapses was also noticed after noise exposure, although the ribbon count was done at only one time point (10 days) after the noise exposure (Lin et al., 2011). Interestingly, the SGN loss evaluated 2 years later in guinea pigs was much less than the ribbon loss measured 10 days after the noise, suggesting that the damage to the ribbon

synapses in guinea pigs might be reversible and ribbon synapse self-repair could be the reason for the reduced loss of SGNs. However, it is hard to concur with this, especially since no data of ribbon synapse repair were available from the guinea pig study (Lin et al., 2011).

Also, there were no data showing the effect of ribbon synapse damage by low level noise exposure on hearing function. Since the ribbon synapse has an important role in fast neural transmission and signalling synchronization (Khimich D et al., 2005; Buran et al., 2010), the temporal processing in the cochlea is expected to be deteriorated, although no data available on this issue.

Therefore, my present study further examines if and how much the ribbon synapse can be self-repaired after noise exposure in guinea pigs, and if and how the noise impacts the hearing function, with a focus on temporal processing in both mice and guinea pigs.

1.2 Background

1.2.1 Cochlear Innervation to Hair Cells

There are the two types of receptor cells in the cochlea of mammals: outer hair cells (OHCs) and inner hair cells (IHCs) (Figure 1). OHCs are innervated by type II SGNs, while IHCs are innervated by type I SGNs. OHCs are known to amplify sound, because they possess a motor protein called prestin, however the functional role of type II SGN innervations to OHCs is not clear. On the other hand, it is

understood that the innervation from Type I SGNs to IHCs is the main pathway for information delivery from the cochlea to the central auditory system.

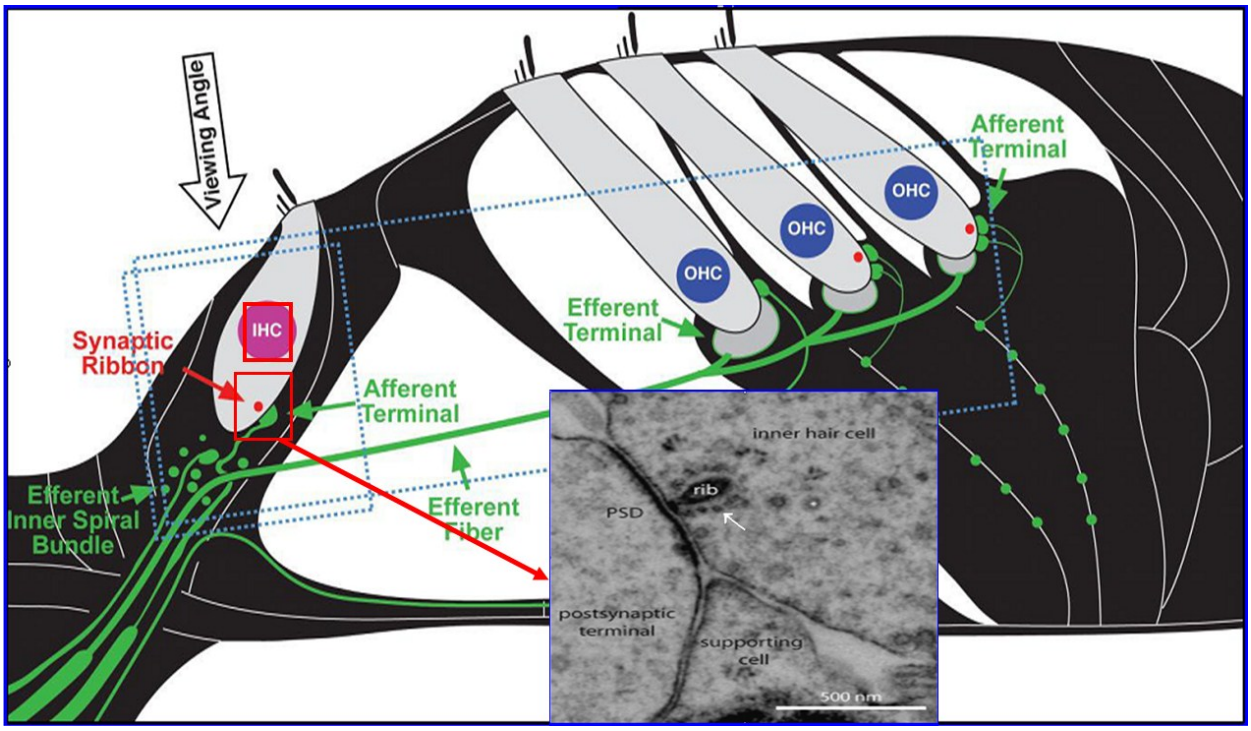

Figure 1. Schematic of the cochlear sensory epithelium showing inner and outer hair cells and their afferent innervations, with inset diagram showing the ribbon synapse in greater detail (Kujawa et al., 2009, Nouvian, 2006).

1.2.2 Ribbon Synapses

The ribbon synapse is between the IHCs and SGNs. It is called a ribbon synapse because of the existence of a bar structure called ribbon (Figures 1 and 2) (Fuchs et al., 2003; Fuchs, 2005; Sterling and Matthews, 2005; Moser et al., 2006; Moser et al., 2006; Nouvian et al., 2006; Schmitz, 2009). Synaptic ribbons are anchored

to the active zone in the presynaptic membrane in mature IHCs (one ribbon per active zone). Ribbon synapses are understood to exist in mammalian cochleae and vestibular organisms, and in retinas (Schmitz, 2009). Although conventional synapses may also exist in the mammalian cochleae, normally ribbon synapses are dominant (Francis et al., 2004).

The ribbon consists of several proteins, including RIBEYE, Bassoon, Picolo, transcriptional co-repressor C-terminal binding protein 1 (CtBP1), and nuclear co-repressor CtBP2 (Schmitz, 2009). RIBEYE has an exclusive aminoterminal, protein-rich A-domain with no identical proteins anywhere else; however RIBEYE's C-terminal B domain is mostly identical to the nuclear co-repressor protein CtBP2 (for ribbon in IHCs) (Schmitz et al., 2000; Schmitz, 2009). The RIBEYE A-domain appears to have a structural function, while RIBEYE B-domain seems to work in interaction with other structures, such as neurotransmitter vesicles because it is facing out toward the cytoplasm (Schmitz et al., 2000; Magupalli et al., 2008; Alpadi et al., 2008; Schmitz, 2009). Bassoon has a major role in the anchoring of the ribbon to the presynaptic active zone (Dick et al., 2001; Dick et al., 2003; Khimich et al., 2005)

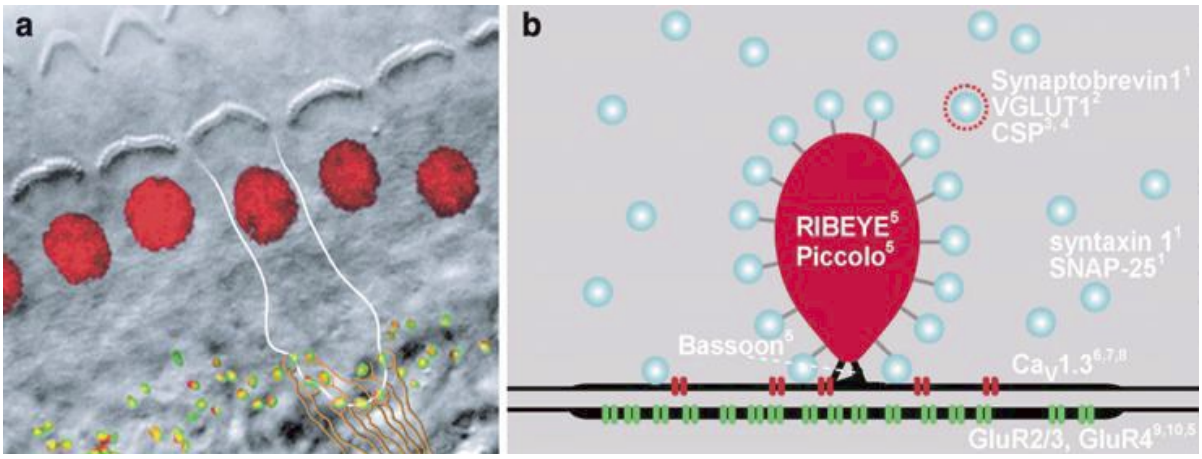

Figure 2. Montage of a Nomarski image and a confocal microscopy for IHCs and ribbon synapse. A: IHCs and the active zone where ribbons are located; also showing convergent afferent innervations. B: Ribbon proteins, and how ribbon is anchored to the active zone. (Nouvian et al, 2006).

The functional role and underlying mechanisms of ribbon synapses are not fully clear. However, based on the available data, ribbon synapses are capable of quick and continuous neurotransmitter release in response to graded changes of membrane potential in receptor cells, as well as in the ongoing recycling of exocytosis and endocytosis (Fuchs, 2005; Moser et al., 2006; Moser et al., 2006; Schmitz, 2009). The fast release of glutamate by ribbon synapses is due to the fact that a group of neurotransmitter vesicles (called readily releasable pool) are docked on ribbons and close to the presynaptic calcium channels; the fast triggered release of these vesicles is probably realized by this structure feature (Schmitz, 2009). Due to this fast release at the onset of stimuli, each single synaptic ribbon

can drive the auditory nerve fibre to fire up to 1000 spikes/s (Johnson, 1980; Buran et al., 2010).

The functional specialities of ribbon synapse in temporal resolution are supported by the temporal processing deterioration observed in the cochlea of mice with Bassoon gene mutation, which mainly causes loss of synapse-anchored ribbons at the presynaptic active zones of IHCs in the cochlea (Buran et al., 2010). Consequently the size of a readily releasable pool of IHC synaptic vesicles, and IHC Ca2+ current were reduced (Brandt et al., 2003; Buran et al., 2010), leading to a reduced exocytosis at the ribbon synapses, especially at the onset of the stimulus. Functionally, poor onset coding and an elongation in the first-spike latencies in auditory nerve fibre were seen without changes in auditory sensitivity (Buran et al., 2010). Therefore, the importance of ribbon synapses in the temporal processing in the cochlea is evident. Accordingly, damage to ribbon synapses would potentially cause deterioration in the temporal resolution of the cochlea.

1.2.3 Noise-Induced Damage of IHC-SGN Synapses and SGN Death

Noise-induced SGN lesions are known to begin at the synapse between IHCs and SGNs. The transmission across this synapse is mediated by glutamate, and the acoustic lesion to the postsynaptic structure is mostly caused by the glutamate excitatoxicity due to the over-release of glutamate (Puel et al., 1998; Puel et al., 2002; Fuchs et al., 2003). As AMPA receptors are glutamate receptors that mediate the fast transmission, it is widely accepted that the toxic effect on the postsynaptic nerve terminal is mediated by AMPA receptors (Ruel et al., 2007).

The noise damage to the IHC-SGN synapses begins with the loss of ribbon in IHCs, which might be associated with damage to and loss of postsynaptic terminals (Kujawa & Liberman, 2009). The terminal retraction increases the distance between the presynaptic zone and receptors and leads to deterioration in the trophic support from hair cells and supporting cell (Kujawa & Liberman, 2009). Accordingly, a slow-developing death of SGNs was seen two years after the noise exposure in mice, suggesting that a large portion of damaged synapses were not re-established (Kujawa & Liberman, 2009). Such SGN death was not reported in many previous studies in which the neuronal and synapse damage of the cochlea by noise was reported as “reversible” (Puel et al., 1998; Pujol and Puel, 1999). The discrepancy in results is largely due to the fact that these earlier studies were not quantitative and did not track the long term survival of SGNs (Puel et al., 1998; Pujol & Puel, 1999).

Particularly in the mouse study conducted by Kujawa & Liberman (2009), a slowly, progressive but massive (~50%) loss of SGNs was found in the cochleae two years after a low-level, brief noise exposure that did not cause permanent threshold shift. It is of interest to notice that the ribbon loss, and probably the post-synaptic terminal that remained unrepaired, is correlated to the percentage loss of SGNs identified two years later.

The result from the mouse study raises significant concerns about the damage induced by low level noise to ribbon synapses and silent death of SGNs. If this occurs in humans, the regular auditory tests would not be sensitive to detect such damage. However, before this can be generalized to humans, such silent SGN death should be verified across different species of experimental animals. Based upon the preliminary data in our lab and reports from others in guinea pigs (Lin et al., 2011), noise-induced late onset SGN death reported in mice does occur in guinea pigs, but at a much reduced scale (~20% at the most severe region) (Lin et al., 2011). Unlike what was documented in mice, the initial loss and recovery of the ribbon in the cochlea of guinea pigs had not been detailed after noise exposure; rather, the ribbon count was reported only at one time point (10 days after noise exposure). Since much less SGN death was seen two years later, presumably the damaged ribbon synapses must have been largely re-connected in guinea pig cochlea if the initial loss of ribbon is comparable to what was seen in mice.

However, no data of ribbon count changes were available from guinea pigs to see the recovery in ribbon numbers after noise exposure.

Furthermore, we do not know what impact the noise-induced ribbon synapse lesion after noise has on hearing function. Since each IHC receives convergent innervations from SGNs, in a ratio of 1:16-25, partial loss of post-synaptic terminals will not impact hearing sensitivity. However, the loss of ribbons and neural terminals may impact coding functions of the cochlea at suprathreshold levels. Given the fact that the ribbon synapse is critical for temporal processing, it is very possible that noise exposure at low levels affects temporal resolution and neural transmission at ribbon synapses. However, no such information was available in either mice or guinea pigs.

1.3 Hypotheses and Objectives

The first objective of the study is to investigate the changes of ribbon counts after the noise exposure in order to determine if the repair of ribbon synapses can account for the reduced loss of SGNs in guinea pigs. If initial loss of the ribbon is comparable between guinea pigs and mice, a better repair of synapses should be the reason for reduced loss of SGN in guinea pig as compared with mice.

The second objective of the study is to investigate the possible impact of the noise-induced lesion of IHC-SGN synapses on the temporal processing of the cochlea in both mice and guinea pigs. I focus on the temporal processing because of the fundamental role of ribbon synapses in the high temporal resolution of the

cochlea. The hypothesis is that the damage to the IHC-SGN synapses can reduce the temporal processing even with undetectable change in auditory sensitivity.

1.4 Justification of the Study

Noise-induced hearing loss is a concern of a large portion of the general population in our society because we are frequently exposed to loud noise in our daily life. If the damage to SGNs caused by noise that doesn't cause permanent threshold shift was found to occur in human cochlea, the current noise control strategies, including safety standards, should be re-assessed. The possibility of noise-induced SGN damage and death without hearing loss is indirectly indicated in the aging population (Makary et al., 2011). This is supported by two facts: firstly, it is known that older people often experience reduced auditory comprehension, especially in noisy environments (Parthasarathy et al., 2010), even though they do not have significant reductions in auditory sensitivity; and secondly, the deterioration of auditory perception is partially due to the deterioration in temporal processing (Simon et al., 2004; Gordon, 2005; Rajan and Cainer, 2008; Grose et al., 2009; Fogerty et al., 2010; Grose et al., 2010; Walton, 2010). Considering its role in temporal processing, the first locus responsible for the temporal processing deterioration along the ascending auditory pathway is likely to be the ribbon synapse between IHCs and SGNs. The subclinical damage by accumulative long-term exposure to low level noise and other hazardous causes under sub-clinical level may reduce the temporal resolution of the cochlea.

Therefore, there is an urgent need to further verify whether the noise-induced lesion to ribbon synapses can be generalized across different species. If this is the case, then it will be important to see whether and how such damage impacts hearing function.

CHAPTER 2 MATERIALS AND METHODS

2.1 Subject and Schedule

This experiment was carried out at young adult animals, including 25 albino guinea pigs (2-4 months of age) and 18 C57BL/6J mice (5-16 weeks of age). Guinea pigs were provided by Charles River Co. Limited, and mice were obtained through in-house breeding. The guinea pigs were used because they are commonly used in auditory research; also they have much larger cochlea than mice. On the other hand, the C57BL/6J mice were used for preliminary study to see the impact of low level noise on temporal processing. The animals were screened using an otoscope to exclude any abnormalities in external ear canals and middle ears. Following that, frequency specific auditory-evoked brainstem response (ABR) was used to evaluate the hearing threshold. Only those that passed both the otoscopic screening and ABR test were used in the next steps.

In the experiment using guinea pigs, the animals were divided into the no-noise control group (n=10) and the noise group (n=15) which was further divided into 3 subgroups according to the time of end-point tests (4, 4, and 7 animals for 1 day, 1 week, and 5 weeks after noise exposure, respectively). The noise exposure was given after baseline ABR for hearing threshold. At each end time point, tonal ABR was repeated to test both hearing threshold and temporal processing, and compound action potential (CAP) was recorded as an end-point test via round window electrode before the animals were euthanized for morphology. CAP was

recorded to test the temporal processing and I/O functions at high frequency region.

In the experiment on mice, after the screening ABR test with subdermal electrodes, a pin electrode was implanted in the skull of each mouse to ensure better signal-to-noise ratio for ABR. The pin electrode was determined to be stable for 2 weeks after implantation. The ABR tests were repeated before, and at different times after the noise exposure until the animals were euthanized. The ABR in mice was recorded to evaluate hearing threshold and temporal processing. The mice were divided into 2 groups: group (1) without electrode implantation for auditory sensitivity test (n=6); and group (2) with electrode implantation for temporal processing test (n=12). The first group was tested for tone-burst ABR before, 1 day and 5 weeks after the noise exposure. The second group was further divided into 2 subgroups according to the skull electrode stability: the first subgroup (n=6) was tested with paired-clicks before, 1 day and 1 week after the noise exposure; the last subgroup (n=6) was tested also with paired clicks but at one time point: 5 weeks post-noise. All animals were euthanized after their final testing.

The noise impact on cochlea was tracked up to 5 weeks after noise exposure because in the mice the ribbon count measured one month after noise exposure didn't differ from the ribbon count measured 8 weeks after noise exposure. Also,

the percentage of ribbon loss measured one month after noise exposure roughly corresponds to SGN loss identified 2 years later (Kujawa & Liberman, 2009).

2.2 Electrode Implantation

For CAP recording from guinea pigs, an electrode was made of 0.007mm silver wire coated with Teflon, with only the tip of the wire being exposed. To reduce the chance of the tip damaging the round window membrane, the exposed tip was coiled under microscope by using a small forceps. The animal was anesthetised using ketamine +Xylocine (40-60 mg/kg +10 mg/kg respectively i.p.) and was kept on a thermostatic heating pad to maintain the body temperature at 38.5 degrees C during the surgery and recording. Injection of local anaesthetic (marcaine) was done before an incision of 2 cm was made behind the ear. The connective tissue and muscles were then retracted to expose the mastoid of the bulla. A small hole of 3-4 mm diameter was made through the mastoid to expose the round window of the cochlea. Under a surgical microscope, the tip of the silver wire was placed gently into the round-window niche to make contact with the round window membrane. Then the wire was fixed and the hold on the mastoid as sealed with dental cement. The other end of the wire was connected to a pin connector, which was then connected with recording cable. Finally, the wound was sutured and the animal immediately placed in the sound booth for recording.

For ABR recording from mice, a pin electrode was implanted under general anesthesia with ketamine + xylocaine 60-80 mg/kg and Rompun 10mg/kg i.m.

The mouse was kept on a thermostatic heating pad to keep the body temperature at 38.5 degrees C when anesthetised. Marcaine was used as a local anaesthetic. After the local anesthesia, a 4 mm circle of the skin was cut off at the top of the skull. A pin connector with a 1.5 mm length of naked silver wire was prepared. After cleaning the exposed area of the skull, a 0.2 mm diameter hole was drilled, and the silver wire from the pin was inserted via the hole and the pin was fixed on the skull with dental cement. Only one electrode was implanted into the skull for recording input, the other two electrodes (ground and references) remained subcutaneously.

2.3 Recording of Physiological Responses

The physiological responses that were recorded were ABR and CAP. In the mouse, ABR waves come from different generators: wave I represents the summed activity of the auditory nerve, while wave II represents the globular bushy cells in the cochlear nucleus, and wave III response is mainly from the inferior colliculus in the brainstem. While the CAP response is mainly dominated by the summed activity of the auditory nerve, the CAP has a higher signal to noise ratio, which makes it more sensitive.

During recording the animal was anaesthetized with the same agents as specified for the surgery or with inhalation of isoflurane. During the recording, the animal was put on a thermostatic heating pad to maintain the body temperature at 38.5 C.

Tucker-Davis hardware and software was used to generate all stimulation signals and record all the responses.

For threshold evaluations, the tone burst-evoked CAP and ABR tests were done at octave steps from 1 to 32 kHz. For each frequency, the threshold was determined through response recording across the intensity range from 90 to 0 dB SPL in 5 dB steps. The evoked responses were amplified (20X), sampled from the electrodes, and averaged over 1000 times for ABR and 200 for CAP (due to the larger signal to noise ratio) with a stimulus repetition rate of 11.1/sec. The responses were band-pass filtered between 100-3000 Hz.

To evaluate cochlear temporal processing (in both CAP and ABR), a regime involving the use of paired clicks was used (Figure 3). The time-stress was applied by varying the intervals between the two clicks (between 20 and 1 ms). Then, the ratio was calculated between the amplitudes of CAP to second click at a particular ISI and that at the longest ISI (20ms). The clicks were presented at several intensity levels from 10 to 50 dB above the click ABR threshold.

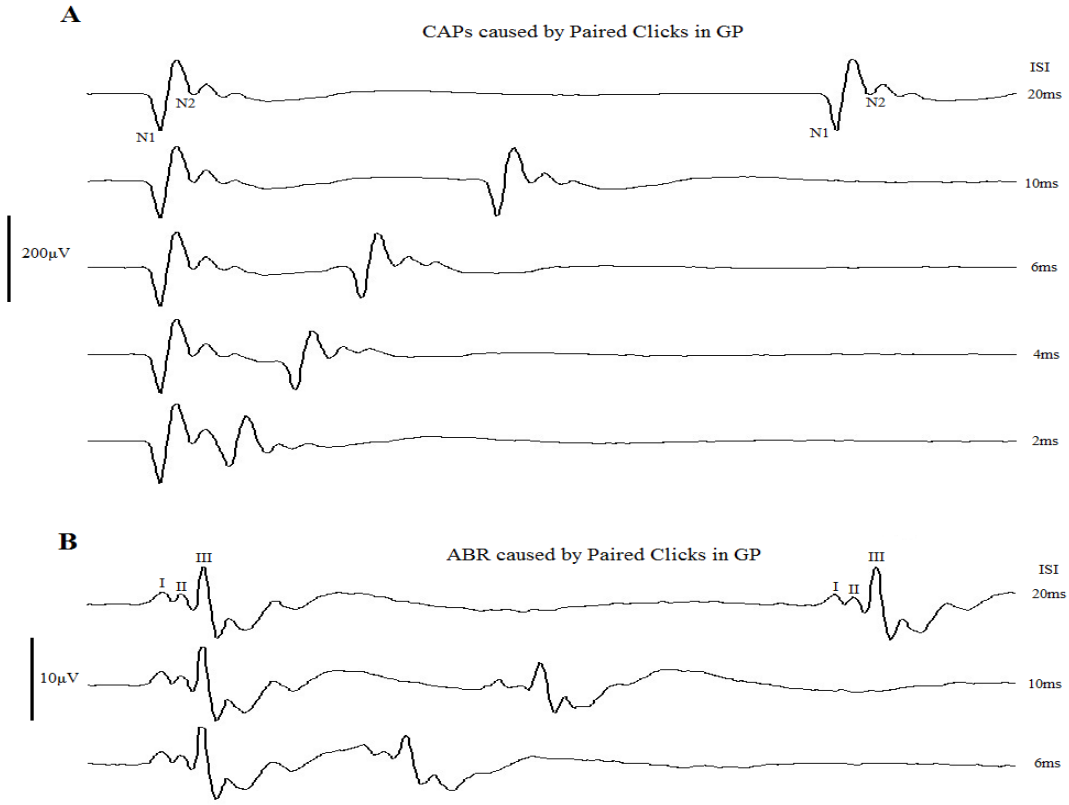

Figure 3. CAP (A) and ABR (B) waveforms evoked by paired clicks of varied ISIs. The time-stress stimulation was done by varying the intervals between the two clicks (between 20 and 1 ms). The ratio was calculated by measuring the amplitudes of second click (from 20-2 ms ISI in CAP and from 20-6 ms in ABR) against the amplitude of second click at longest ISI (20ms). The ratio for ABR could not be done for ISIs less than 6 ms because of the large overlaps in ABR waves. The response amplitude is reduced with decreasing ISI. N1 represents summated action potentials of spiral ganglion neurons; N2 represents the response from cochlear nucleus neurons. Wave I represents summed activity of the auditory nerve, Wave II represents the response from globular busy cells in the cochlear nucleus, Wave III represents the response from inferior colliculus in the brainstem.

2.4 Noise Exposure

During noise exposure, the animal was awake and could freely move to access food and water. The dimensions of the cage, which was covered with metal mesh, were 20 x 24 x 40 cm. Two speakers, one a low-frequency subwoofer and the other a high-frequency tweeter, were used for noise exposure to ensure a relatively flat spectrum of broadband noise up to 25 kHz. The noise exposure was monitored using a sound level meter (Larson Davis 824), and the exposure was controlled to 103 dB SPL for 2 hours.

2.5 Morphology and Immunohistology

Each guinea pig was decapitated following an overdose of anaesthesia (pentobarbital 100 mg/kg. i.m.). Then, the epithelial whole mounts of cochlear organ of Corti were prepared after fixation and decalcification. The whole guinea pig cochlea (normally lengthened ~20 mm with ~3600 IHCs) was dissected into ~5-10 pieces and then immunostained for CtBP2. The ribbon counts were measured at 15 points across the whole mount according to the distance from the apex of the cochlea. The frequency map of these points is calculated as in ~half octave steps from 0.1 to 48 kHz based upon the frequency-distance map of the guinea pig cochlea previously reported (Viberg & Canlon, 2004). At each location, the basilar membrane is viewed under microscopy in 2-4 segments, 0.24 mm each containing ~28-33 IHCs. The total number of IHCs (as CtBP2 stained IHC nuclei)

and ribbons (as CtBP2 stained red spots) were counted using ImageJ software in each segment to get the mean number of ribbons in each IHC.

2.6 Data Analysis

2.6.1 To Evaluate the Threshold:

For both guinea pigs and mice, the ABR threshold to tone burst and click were measured. Threshold was judged as the lowest SPL at which a repeatable response was visible. Responses to CAP I/O functions on amplitude were also evaluated before and 5wks after noise exposure.

2.6.2 To Evaluate the Temporal Processing

The ratio of CAP (N1) and ABR (wave III) between those responses obtained at the targeted ISI and the longest one was measured as a function of ISI to be the indicator of temporal processing. The ratio function for CAP was compared across different time points after the noise exposure in guinea pigs (1 day, 1 wk, and 5 wks after noise) and compared to that obtained from no-noise control animals. For ABR the ratio function was calculated for 1 wk and 5 wks after the noise and compared to no-noise control. No data are reported for ABR ratio data at 1day post noise, because the amplitude of wave III is largely reduced at that time and is too small for a reliable measure of the peak amplitude. The latency of CAP (N1) of the second click response was also measured at different ISI's.

2.6.3 To Evaluate the Change of Ribbon Number After Noise Exposure in Guinea Pig

The number of ribbons was counted across different time points after noise exposure in guinea pigs (1 day, 1 wk, and 5 wks after noise) and compared to the no-noise control. The ribbon count was calculated as the number of ribbons per IHC, and was normalized as percentage change using the control value as 100%.

2.6.4 The Statistical Analysis

One-way analysis of variance tests were used for multi-comparisons to see any significant difference between control group and post-noise groups, followed with Tukey's HSD (Honestly Significant Difference) test to test post-hoc differences. T-test was used for statistical comparison between two groups (the control group vs. the 5 wks post-noise group). If the p-value < 0.05, the result was statistically significant. Standard errors were measured for the ratio and standard deviation for the rest. P-values for the ratio were calculated after converting the ratio to arcsin values because the ratio values need to be normally distributed.

CHAPTER 3 RESULTS

3.1 Thresholds Fully Recovered 5 weeks After Noise Exposure in both Guinea Pigs and Mice

After the brief noise exposure (103 dB SPL) for mice and guinea pigs respectively, there was large elevation of the ABR threshold one day after the exposure, which gradually recovered. The audiogram curves (shown as tone-burst ABR thresholds in Figure 4 A and B) obtained 5 weeks after the noise were largely overlapped with that of the controls in both species across the frequency range tested (1 kHz-32 kHz). One way ANOVA test was done for each frequency group, followed with Tukey's HSD (Honestly Significant Difference) test to test post-hoc differences. The Tukey's HSD (Honestly Significant Difference) test mostly shows that control group is statistically different from 1day post-noise group but not different from 1week and 5 weeks post-noise group, this was seen in both guinea pig and mice. The click-evoked ABR threshold (Figure 4 C and D) was elevated one day after noise, largely recovered one week, and fully recovered 5 weeks after the noise exposure in both mice and guinea pigs. The threshold change in click-evoked ABR (Figure 4 C and D) showed a similar trend to the tone-burst ABR.

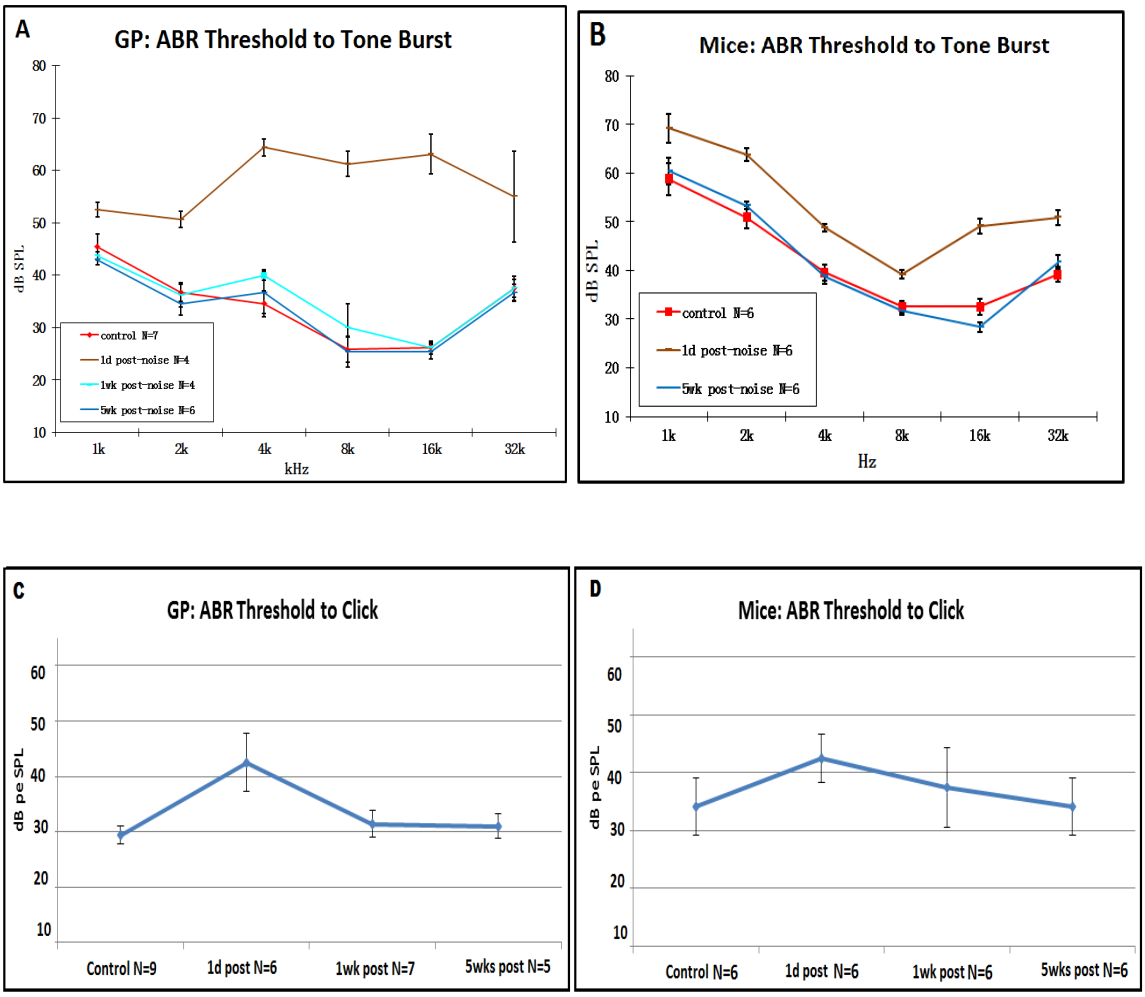

Figure 4. Changes in ABR thresholds. A: Changes after noise exposure in ABR threshold to tone in guinea pigs. B: Changes after noise exposure in ABR threshold to tone in mice. C: Changes after noise exposure in ABR threshold to click in guinea pigs. D: Changes after noise exposure in ABR threshold to click in mice. A full recovery was seen five weeks post-noise exposure. One way ANOVA test was done followed with Tukey's HSD (Honestly Significant Difference) test to test post-hoc differences. For graph A, one way ANOVA was tested for each frequency group followed with Tukey's test; the control is significantly different from one day post noise but not different from 1week and 5week post-noise (the statistical data are summarized in table 1). For graph B, one way ANOVA was tested for each frequency group followed with Tukey's test; the control is significantly different from one day post noise but not different from 5week post-noise, but at 1kHz there is no significant differences between controls and post-noise groups (the statistical data are summarized in table 2). For graph C, One Way ANOVA was tested for 4 groups (control, 1day, and 5 weeks post-noise exposure), the control was significantly different from one day post-noise but not different from one week and 5weeks post-noise (statistical data summarized in table 3). For graph D, there is no significant difference between the 4 groups (the statistical data are summarized in table 4)

Table.1

Graph (A): One Way ANOVA for Guinea Pig ABR Threshold to Tone Burst. The analysis was done for each frequency at control, 1day, 1week and 5 weeks post-noise exposure	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
Frequency: 1kHz	5.45	0.009	3 & 16
Frequency: 2kHz	11.38	<0.0001	3 & 16
Frequency: 4kHz	19.03	<0.0001	3 & 16
Frequency: 8kHz	27.70	<0.0001	3 & 16
Frequency: 16kHz	89.79	<0.0001	3 & 16
Frequency: 32kHz	4.79	0.014	3 & 16

Table.2

Graph (B): One Way ANOVA for Mice ABR Threshold to Tone Burst. The analysis was done for each frequency at control, 1day, and 5 weeks post-noise exposure	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
Frequency: 1kHz	3.42	0.06	2 & 15
Frequency: 2kHz	18.69	<0.0001	2 & 15
Frequency: 4kHz	15.93	<0.0001	2 & 15
Frequency: 8kHz	19.21	<0.0001	2 & 15
Frequency: 16kHz	57.07	<0.0001	2 & 15
Frequency: 32kHz	16.98	<0.0001	2 & 15

Table.3

Graph (C): One Way ANOVA for Guinea Pig ABR Threshold to Click was tested for 4 groups (control, 1day, 1week and 5 weeks post-noise exposure)	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
To click	23.55	<0.0001	3 & 22

Table.4

Graph (C): One Way ANOVA for Mice ABR Threshold to Click. The analysis was done for 4 groups (control, 1day, 1week and 5 weeks post-noise exposure)	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
To click	3.28	0.042	3 & 20

3.2 CAP Amplitude was not Fully Recovered at High Sound Level After 5 weeks Post-Noise Exposure in Guinea Pig

CAP input/output (I/O) function was measured at 16 kHz to see if the CAP amplitude at 16kHz recovered 5 weeks after noise exposure. The result shows that the maximal amplitude at high sound levels tested (90 dB SPL) remained low 5 weeks post-noise exposure (Figure 5), while the threshold was fully recovered, as in the ABR. The t-test shows no significant difference between control vs. 5 weeks after noise group; however, the curve trend shows that the amplitude is not recovered at a higher sound level, where the depression of the maximal CAP amplitude was found to be only 23%.

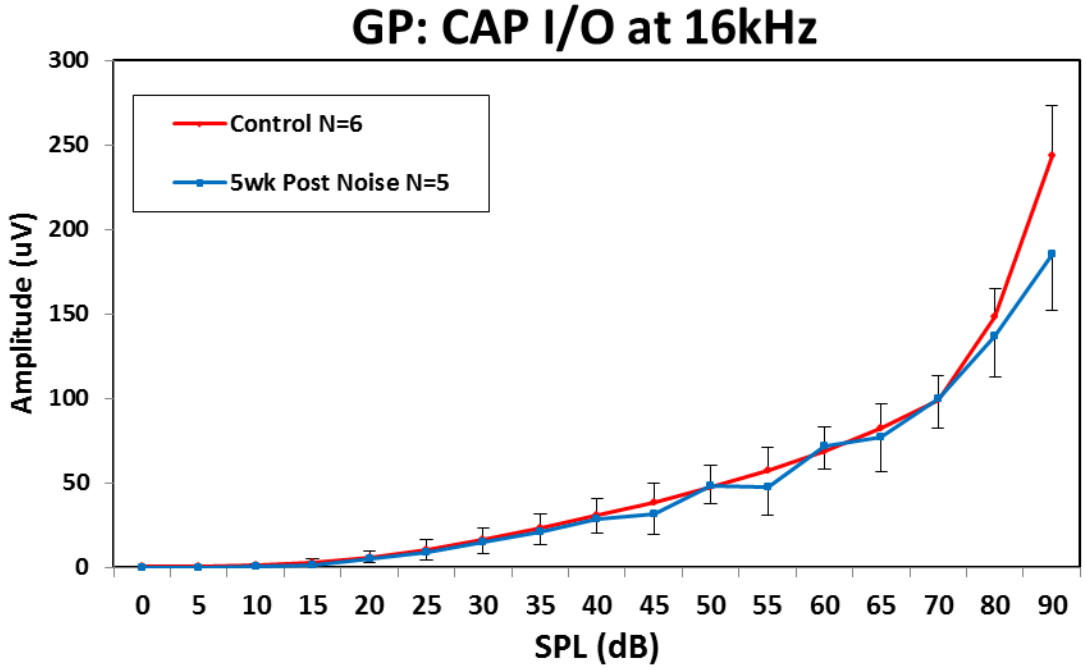

Figure 5. CAP I/O function at 16 kHz in guinea pigs did not fully recover after 5 wks post-noise exposure, suggesting the loss of auditory channels. T-test shows no significant changes between the two groups; however, the trend shows amplitude reduction at higher sound level. The depression of the maximal CAP amplitude at 90dB SPL is 23%. T-test was used to compare control vs. 5wk post-noise at 80 and 90 db; there is no significant difference between control group and 5wk post-noise group (the statistical data are summarized in table 5).

Table.5

T-test for Two Groups Comparisons for CAP I/O at 16kHz, control vs. 5wks post-noise at 80 and 90 dB SPL	**t-statistic**	**p-value**	**Degree of freedom**
80 dB SPL	0.38	0.712	7
90 dB SPL	1.33	0.220	8

3.3 Ribbon Count Change after Noise is Suggesting a Large Repair in Guinea Pigs

The number of ribbons was counted at different cochlear regions (from apical to basal) for control and then 1 day, 1 wk, and 5 wks after noise exposure (n=4 for control and 1 day; n= 8 for 1wk group; and n= 7 for 5 wk group). The impact of noise exposure is more severe in medium and high frequency regions than in low frequency regions. One day after the noise exposure, there was a massive loss of ribbon across all the cochlear regions, less (20%-40%) at low frequency regions and much more loss (60%-70%) at high frequency regions (Figure 6 A & B, and Figure 7 A & B). Unlike what was reported in mice (Kujawa & Liberman, 2009), the initial loss of ribbon synapses was largely recovered 1 week post-noise exposure (e.g. from ~60% loss, to ~25% at region around and above 16 kHz) (Figure 6 A & C, and Figure 7 A & B). Moreover, 5 weeks after noise exposure, the number of ribbons mostly recovered at low frequency regions, but some loss (~15%-20) remained at medium and high frequency regions. The recovered ribbons had been dislocated away from the active zone one week after the noise, but they were mostly located back to the original position 5 weeks after noise (Figure 6 A, C, & D). The ribbon recovery seen 1 week and 5 weeks after noise exposure is evidence for ribbon synapse spontaneous recovery in guinea pigs after noise exposure. This was evident as the change of distance between the ribbon and the nuclei of IHCs.

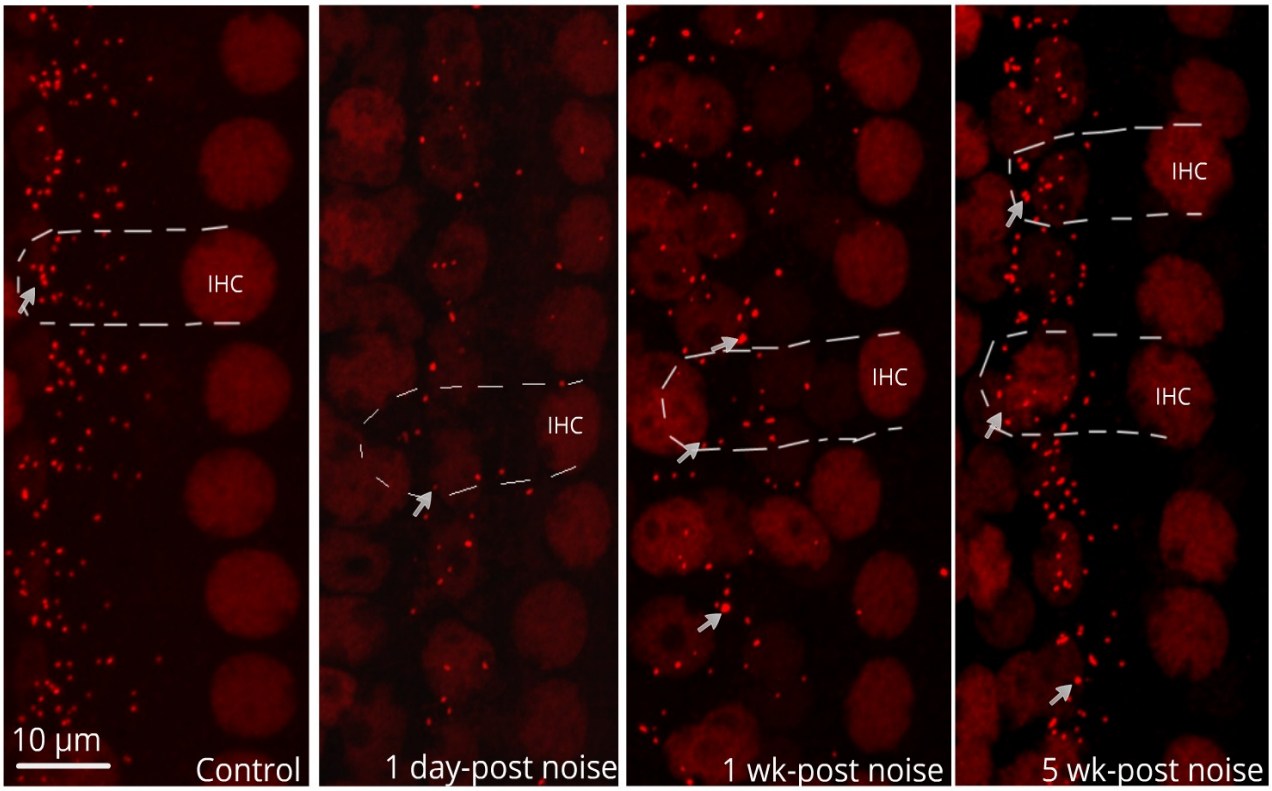

Figure 6. Representative images for noise-induced ribbon changes in 20 kHz region. A significant and quick loss of ribbon was seen shortly after noise exposure, followed with incomplete recovery. Dislocation and increase in size of the ribbons are also seen one week after the noise exposure.

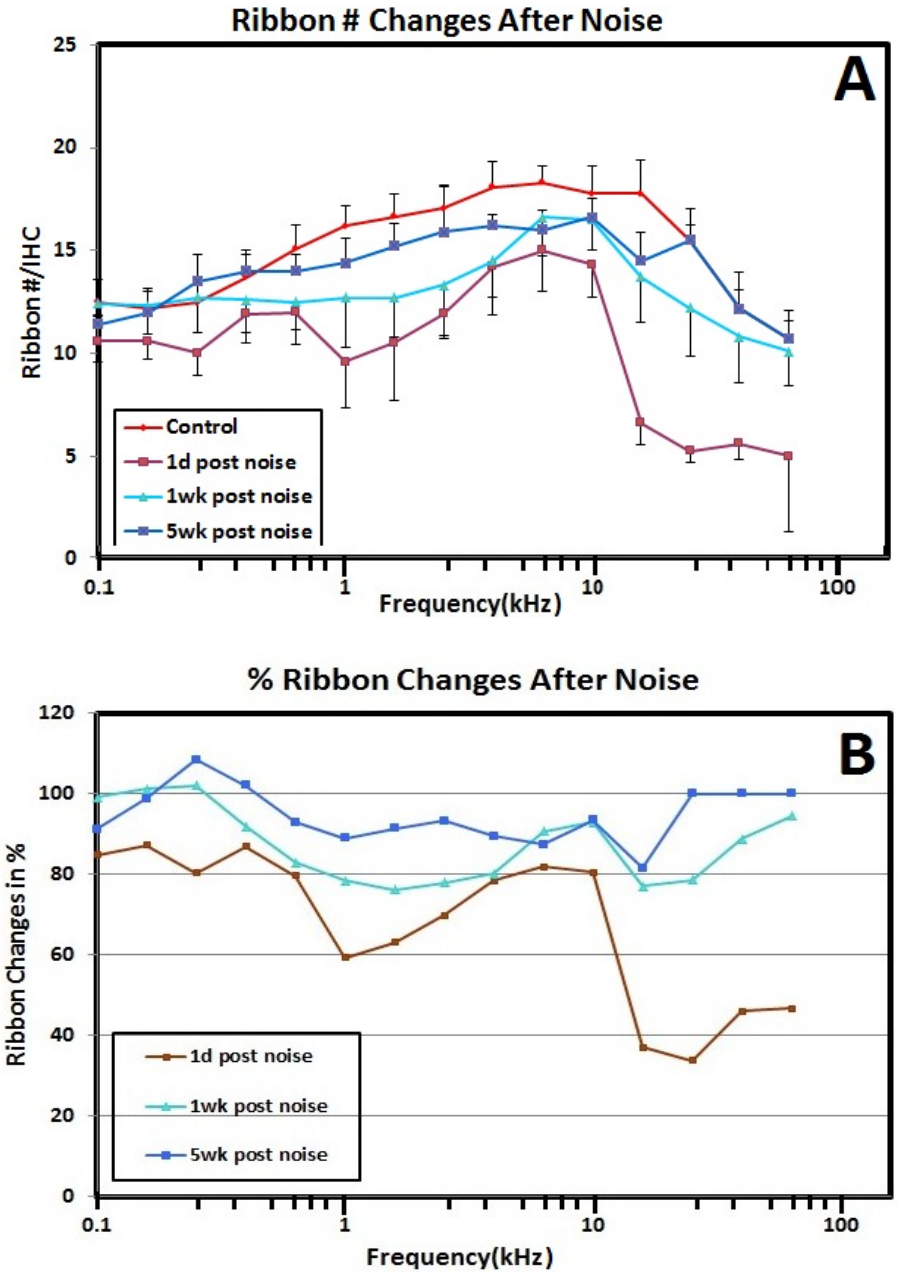

Figure 7. Guinea Pig cochleogram for ribbon loss across different cochlear regions (from apical to basal). A: Ribbon number changes at different time points after noise compared to no-noise control group. B: Normalized using control values as 100%.

3.4 CAP and ABR Amplitude to the Second Click Declined more Quickly with Decreasing ISI after Noise

Temporal processing of the cochlea was evaluated by measuring the ratio of CAP (N1) and ABR (wave III) as peak amplitude of the second click to the amplitude of the second click response at the longest ISI (20ms). The rationale is to see whether or not the amplitude of the 2nd click at shorter ISI is reduced after noise as a result of time-stress stimuli. The ratio for CAP in guinea pigs was severely reduced one day post-noise exposure, started to recover 1 week post-noise exposure, and was largely recovered 5 weeks after noise exposure (Figure 8). The ABR ratio from guinea pigs and mice showed the same trend as seen in CAP from guinea pigs (Figure 9 A & B). One day after noise exposure, ABR wave III amplitude was severely reduced, making it hard to measure the ratio at this time point; therefore, there is no ratio data at 1 day post-noise. The ratio reduction seen in CAP and ABR from guinea pigs and mice is an indication for temporal processing deterioration, despite the full recovery in thresholds. The CAP ratio reduction (at 2ms ISI) seen 1 day was statistically significant compared to control (Figure 8); the significance was verified with one way ANOVA followed with Tukey's HSD (Honestly Significant Difference) test to test post-hoc differences. Furthermore, the significant difference was also seen in ABR ratio for both mice and guinea pigs, also verified with one way ANOVA followed with Tukey's HSD (Honestly Significant Difference) test to test post-hoc differences (Figure 9 A & B).

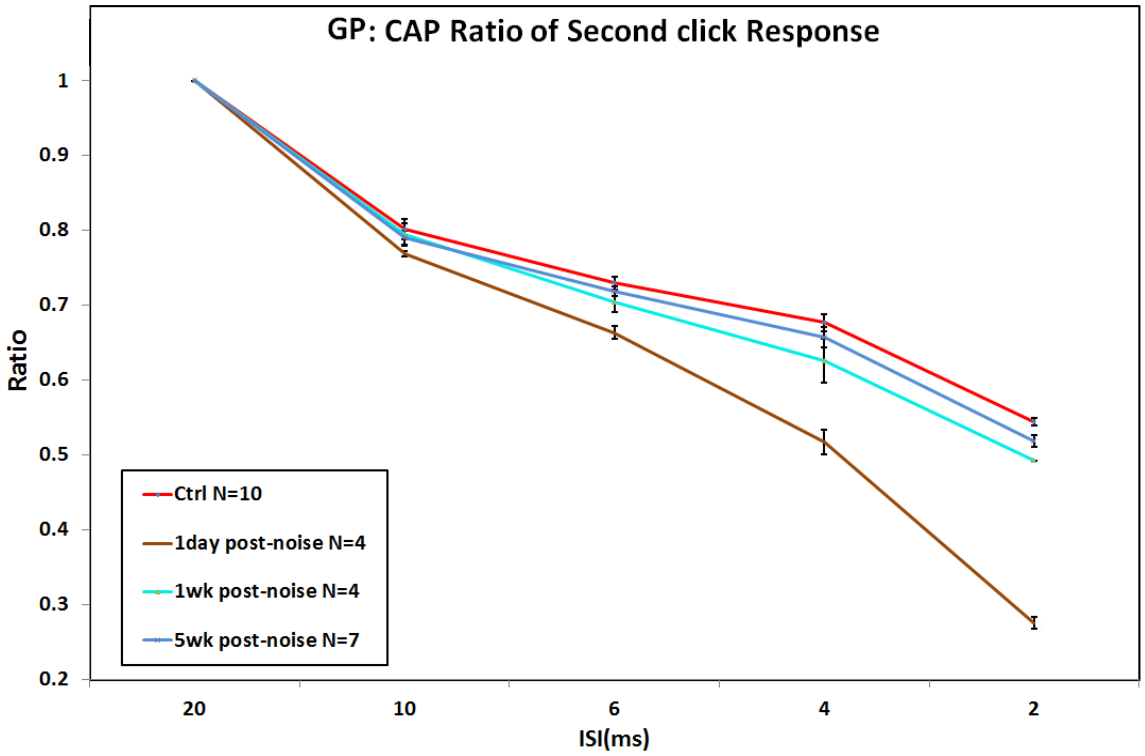

Figure 8. Noise induced changes in CAP response amplitude to 2nd click against ISI in guinea pigs. The ratio is calculated for the amplitude of 2nd click against the amplitude of the second click at the longest ISI (20 ms). The sound level is 60 dB peSPL. One way ANOVA was tested for 2ms and 4ms (ISI) groups followed with Tukey's test; the control is only significantly different from one day post noise but not different from 1week and 5week post-noise at 2ms (ISI) (the statistic data are summarized in table (6)).

Table.6

One Way ANOVA for Guinea Pig CAP Ratio. The analysis was done for 2ms and 4 ms (ISI) at control, 1day, 1week and 5 weeks post-noise exposure	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
2ms	8.98	0.001	3 & 21
4ms	1.26	0.313	3 & 21

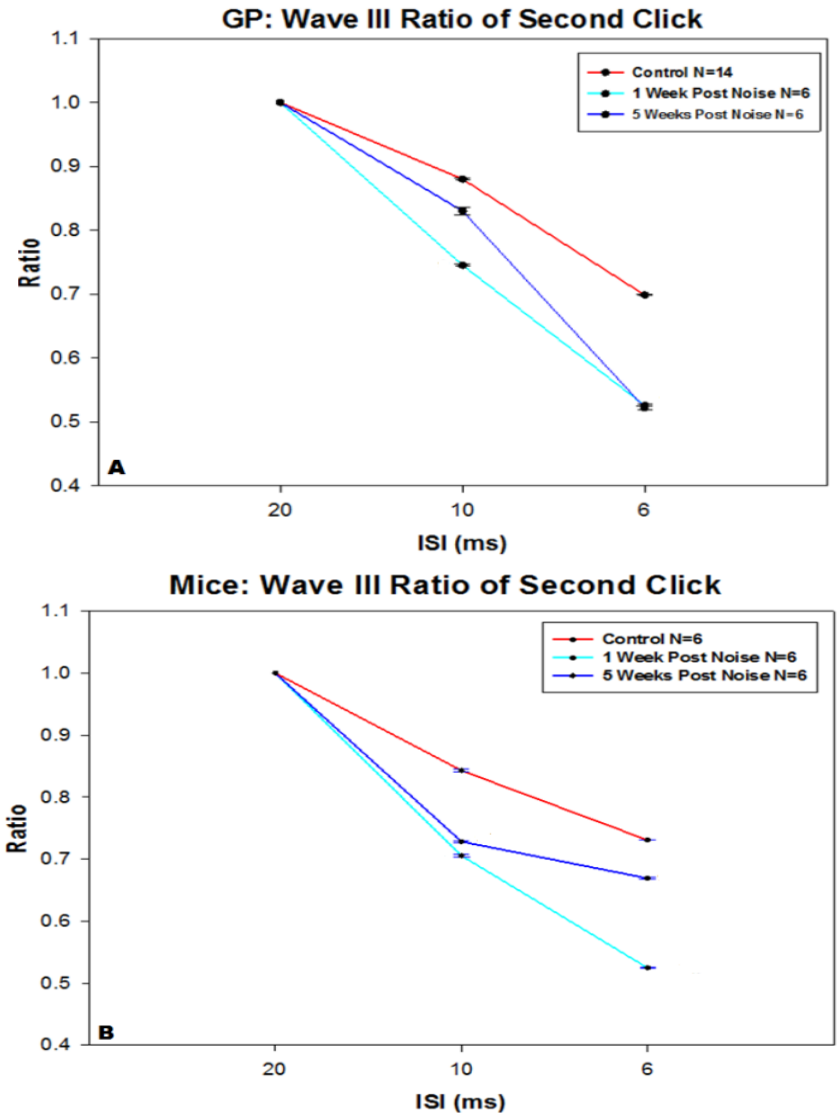

Figure 9. Noise induced changes in ABR wave III amplitude in response to the second click. A: ABR wave III amplitude ratio from guinea pigs. B: ABR wave III amplitude ratio from mice. The ratio is calculated against the amplitude at the longest ISI (20 ms). The sound level is 60 dB peSPL. Significant reduction in the ratio was seen at short ISI after noise by 1 week and recovery but not complete was also seen 5 weeks after noise. For graph A, One way ANOVA was tested for 6ms and 10ms (ISI) groups followed with Tukey's test; at 6ms (ISI) the control is significantly different from one week post noise but not different from 5week post-noise, but no significant difference between groups at 10ms (the statistical data are summarized in table 7). For graph B, One way ANOVA was tested for 6ms and 10ms (ISI) groups followed with Tukey's test;at 6ms and 10ms (ISI's) the control is significantly different from one week post noise but not different from 5week post-noise (the statistical data are summarized in table 8)

Table.7

Graph A; One Way ANOVA for Guinea Pig ABR Ratio. The analysis was done for 6ms and 10 ms (ISI) at control, 1week, and 5 weeks post-noise exposure	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
6ms	8.03	0.002	2 & 23
10ms	2.68	0.09	2 & 23

Table.8

Graph B: One Way ANOVA for Mice ABR Ratio. The analysis was done for 6ms and 10 ms (ISI) at control, 1week, and 5 weeks post-noise exposure	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
6ms	16.41	<0.0001	2 & 15
10ms	4.47	0.03	2 & 15

3.5 Increased CAP Latency of the Second Click Response in Guinea Pigs

Temporal processing of the cochlea was also evaluated by measuring CAP (N1) latency after noise exposure compared to no-noise control group of guinea pigs. The latencies of N1 of the second click response, when ISI is 20 ms and 6 ms, were measured for control, 1 day, 1 week, and 5 weeks after noise exposure. The N1 latency was largely elongated 1 day after noise, started to recover 1 week post-noise exposure, and then fully recovered after 5 weeks post-noise exposure (Figure 10). The delay in N1 latency was severe at shorter ISI (6ms) than at longer ISI (20ms). This also suggests temporal processing declination. In addition, the latency recovery seen 5 weeks after noise is evidence for neural reconnection and synapse repair. The statistical significance was verified with one way ANOVA followed with Tukey's HSD (Honestly Significant Difference) test to test post-hoc differences.

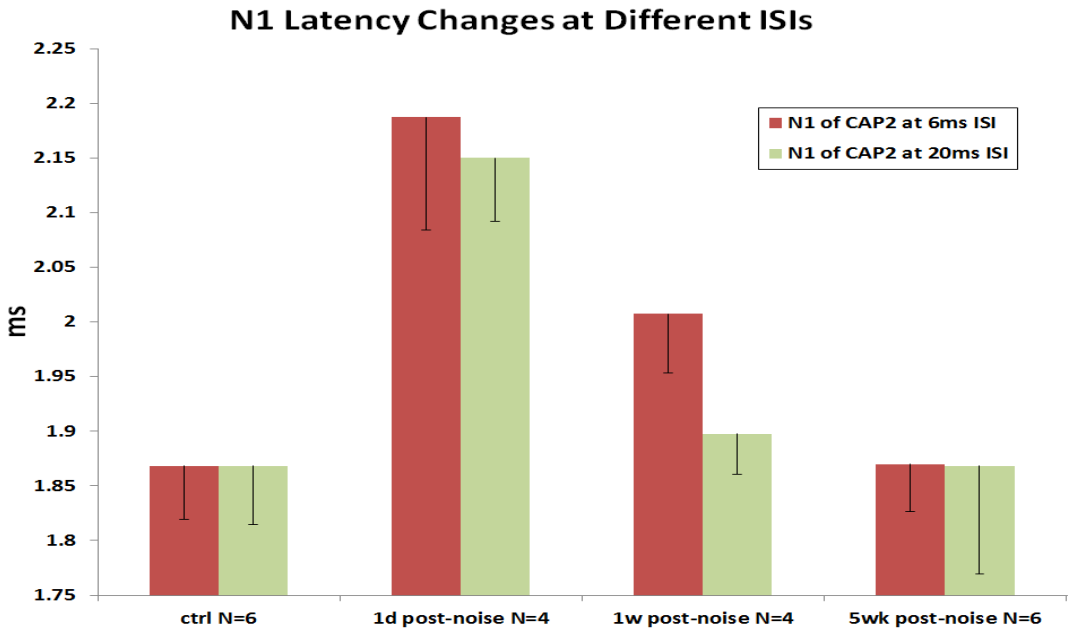

Figure 10. Changes in CAP (N1) latency in response to second click at ISI = 20 and 6ms. The sound level is 60 dB peSPL. The latency was elongated after 1 day post-noise at 6ms and 20ms ISI, and then started to recover 1 week post-noise exposure; latency is almost recovered at longer ISI (20ms). Full recovery in N1 latency at both ISI's was seen 5 weeks after noise exposure. One way ANOVA was tested for multi-comparisons (control, 1day , 1wk and 5wk post noise exposure) followed with Tukey's test; for 6ms (ISI) groups the control is significantly different from one day post noise and 1wk post-noise but not different from 5 wk post-noise. For 20ms (ISI) groups the control is significantly different from 1day post-noise but not different from 1wk and 5wk post-noise (the statistical data are summarized in Table 9).

Table. 9

One Way ANOVA for Guinea Pig CAP Latency of the Second Click Response at 6ms and 20ms (ISI). The analysis was done at control, 1day, 1week, and 5 weeks post-noise exposure	**F-statistic**	**p-value**	**Degree of freedom1 & degree of freedom 2**
6ms	27.00	<0.0001	3 & 16
20ms	16.85	<0.0001	3 & 16

CHAPTER 4 DISCUSSION

4.1 Difference between Mice and Guinea Pigs in Noise-Induced Ribbon loss and the Fate of SGNs

The result of this experiment clearly demonstrated a significant difference in ribbon loss between mice and guinea pigs in response to the noise exposure. In mice, a massive ribbon loss (~50-60%) was seen shortly after noise with subsequent limited repair (~10%) within a week (Kujawa & Liberman, 2009). From our study, the ribbon loss in guinea pigs seen shortly after the noise exposure was comparable to that in mice, but was largely recovered between 1 and 5 weeks post noise; the highest residual loss of the ribbon 5 wks after the noise was less than 20%. Although the post-synaptic terminal was not counted, the recovered ribbon is likely accompanied by the recovery of the synapse. This is supported mainly by two facts. Firstly, the depression of the maximal CAP amplitude was found to be only 23% 5 weeks after the noise at 16 kHz, which is generally consistent with the residual ribbon loss at this time around this region where the highest residual loss of ribbon was found. We specifically tested CAP I/O at this frequency because the maximal ribbon loss was seen at this frequency region and above. Due to the up-spreading of cochlear excitation at the high level (90 dB SPL), all the channels at and above 16 kHz should be recruited. Therefore, the maximal amplitude of CAP at this frequency should be a good indicator for the total channels available in this frequency region, and the depression in maximal

amplitude is likely representing the loss of channels due to the unrepaired synapses. Secondly, the residual loss of ribbon is quantitatively consistent with the SGN loss reported 2 years later after a comparable noise exposure as reported by Lin et al. (2011). This "coincidence" of result can be pleasantly explained as the result of the re-established ribbons and synapses; because otherwise the SGN would have died without synapse to contact IHC.

We did not observe CAP I/O in mice. However, the residual and stabilized ribbon loss reported by Kujawa & Liberman (2009) in mice was 50% one month after the noise, which is also consistent with the long-term loss of SGNs. Therefore, the ribbon count several weeks after the noise appears to be a good indicator for the fate of SGNs.

The reasons behind SGN loss variation between mice and guinea pigs remain unclear. As explained above, ribbon synapse reparability is a potential reason for the difference in the amount of SGN loss between mice and guinea pigs. For this matter, we should consider that the number, shape, and size of ribbon synapses vary across species (Nouvian et al., 2006). As explained in the background section in chapter one, the initial damage of ribbon synapses is likely to be associated with damage and loss of the postsynaptic terminals (Kujawa & Liberman, 2009). Consequently, the terminal retraction will widen the distance between the presynaptic and postsynaptic zone; this would cause deterioration in the IHCs and supporting cells trophic support to the SGN (Kujawa & Liberman, 2009).

4.2 Deterioration in Cochlear Temporal Processing

Considering the role of ribbon synapses for high temporal resolution in processing a broad range of frequencies and intensities with different temporal features, the acoustic damage to ribbon synapses is most likely to have impacted the temporal processing. Therefore, our investigation used time-stress stimulation to functionally test the temporal processing to evaluate the noise impact on hearing function, despite the full recovery in threshold. Our tests (paired-click with varied ISI) were sensitive for such damage, and interestingly the obtained results were consistent with the morphology data. The consistency was seen in both ABR and CAP. The ABR test seems more sensitive than CAP, this was evident by the significant difference between control group and 1week or 5week post-noise groups at longer ISI's (6 and 10ms) in ABR, but in CAP the significance difference was only seen at 2ms ISI. The possible explanation for this is that the CAP is recorded from one ear, while ABR is representing the sound-evoked potential generating from both ears where the ribbon damage is more.

Our results have shown temporal processing declination after noise exposure. The deterioration was severe 1 day post noise, but started to recover 1 week and 5 weeks post noise exposure. The deterioration in temporal processing was evident in the reduction seen in CAP (N1) and ABR (wave III) amplitudes of the second click responses when time-stress stimuli were applied at shorter ISI's. Also, elongation in CAP (N1) latency was seen after noise and was severe at shorter

ISI's. The declination in temporal processing might be due to the partial loss of innervations and the functional changes in the surviving synapses. The declination in temporal processing after noise may occur through three possible mechanisms. First, it could be by the loss of auditory ion channels, as the initial loss of ribbon might be associated with loss in postsynaptic terminals channels at the active zone (Buran et al., 2010). In the present study the CAP amplitude was fully recovered at a low sound level but not at a high sound level (Figure 5), suggesting remaining loss of auditory channels. Secondly, the surviving channels may have been partially damaged so that they functionally cause deterioration in the temporal processing. In our study, we found at one week after noise that the ribbon is floating, which might impact the function of the surviving channels, also the ribbon loss might be associated with reduction in Ca2+ channel density, this might leads to impairment in the trigger of synaptic exocytosis. Thirdly, the repaired channels would have low temporal resolution due to the residual loss of the synaptic ribbons after noise.

4.3 Ribbon Loss and its Role in Postsynaptic Damage

It is not clear at this moment about the nature of ribbon damage caused by noise and the relationship or role of ribbon damage in the lesion at postsynaptic terminals. In the present study and those reported by others (Kujawa & Liberman, 2009; Lin et al., 2011), ribbon loss was observed by immunostaining against CtBP2, the B-domain protein of the RIBEYE. It is not clear if the loss of CtBP2

signal represents the total loss of ribbon, or if ribbon with different degrees of damage exists, which may cause variation in their reparability. Therefore, the ribbon loss measured by CtBP2 would not completely represent or explain the role of ribbon loss in the postsynaptic zone.

As reported (Kujawa & Liberman, 2009) and in our results, the ribbon loss happened to be quick and massive immediately after the exposure. The rapid loss of ribbon was reported to might be associated with loss of the unmyelinated postsynaptic terminals that contacted them, while the myelinated postsynaptic terminals experienced a slow degeneration due to the loss of the neurotrophic support (Kujawa & Liberman, 2009). Therefore, the time-relationship between ribbon loss and postsynaptic damage is not clear.

It is confirmed that the postsynaptic terminal damage after noise is mediated by AMPA receptors and is due to the glutamate excitotoxicity (Puel et al., 1998). Since the ribbon mediates the release of glutamate (Sterling & Matthews, 2005), the initial massive loss of ribbon could be a protective mechanism to protect the remaining terminal from being damaged by excitotoxicity. However, since this has not been proved, it is difficult to clarify the impact of ribbon loss on the postsynaptic zone, whether it is to damage or to protect.

It was suggested by Lin et al. (2011) that the ribbon counts might underestimate the actual damage on post-synaptic terminals. As previously reported (Kujawa & Liberman, 2009; Lin et al., 2011) and in our morphology data, it is evident that

ribbons are dislocated. These dislocated ribbons are more likely to not function properly because they are away from the active zone and from auditory nerve terminals. Therefore, the count of ribbon loss might not represent the loss of terminals.

4.4 Subclinical Noise Damage to Cochlear Afferent in Humans

It was believed for long time that the loss of SGNs is secondary to the loss of sensory hair cells. However, a recent study (Kujawa & Liberman, 2009) has shown that after noise exposure, animals have suffered SGN death even with intact hair cells. This phenomenon of SGN loss with intact hair cells has also been seen in a recent study on human temporal bones (Makary et al., 2011). It is of interest to mention that in the study of human temporal bones, 3 subjects had a history of noise exposure, and 2 of them had more SGN loss than age-matched subjects (Makary et al., 2011). Furthermore, recent studies on humans have shown that elderly subjects often experience decreased auditory comprehension, especially in noisy environments, even though they do not have significant deficits in auditory sensitivity (Simon et al., 2004; Gordon, 2005; Rajan and Cainer, 2008; Fogerty et al., 2010; Grose et al., 2009 Grose et al., 2010; Parthasarathy et al., 2010; Walton, 2010). Also, the research findings in these studies have suggested that a decline in temporal processing might contribute in the deterioration of auditory perception. However, the loci that are responsible for this deterioration are not clear. Previous studies focused more on the contribution from the central

auditory system where other factors, such as cognitive ability, are involved in auditory perception. In our study, we showed that the deterioration of temporal processing occurs in the auditory peripheral sensory organ and is accompanied with damage to ribbon synapses. Therefore, the deterioration in temporal processing seen in elderly people may occur due to unnoticeable damage to ribbon synapses caused by accumulative long-term exposure to low level noise and other possible hazardous factors. Cross-species variation in vulnerability and ribbon damage to noise exposure would weaken this hypothesis, but it is important to remember that these animals were exposed only one time to 2 hrs of low level noise, while humans are often exposed to prolonged loud noise in their daily lives.

4.5 Potential Mechanisms for Threshold Recovery when Ribbon Loss Remains

Due to the fact that IHCs receive convergent innervations from SGNs in a ratio of 1:16-25, this abundance may maintain the sensitivity of hearing after partial loss of the innervation. Also, it is well known that with regular auditory tests, sensitivity thresholds would not change as long as the hair cells are intact (Wang et al., 1997; Kujawa & Liberman, 2009).

OHCs are known to play a role in keeping the sensitivity threshold by providing a mechanical feedback that enhances the movement of the basilar membrane (Wang et al., 1997). This is also a major factor in keeping the threshold unchanged after

the ribbon and neural damage from noise. As reported, OHCs and their ribbon synapses were not affected after the noise impact (Kujawa & Liberman, 2009).

In a carboplatin treated animal (carboplatin causes quick and severe rapid damage to the nerve fibres in the cochlea), neural hyperactivity was seen as an increased driven discharge rate (DDR) after the injection, which suggests a compensation mechanism following the neural damage (Wang et al., 2003). This could also contribute to the threshold recovery after the noise exposure (El-Badry et al., 2007).

The ABR test that is used as a regular test for noise impact is based on a visual judgment of wave III at the lowest SPL, and does not measure the wave amplitude. Considering that the response was recorded across the intensity range from 90 to 0 dB SPL in 5 dB steps, it is very possible that a permanent threshold shift could happen in less than a 5dB difference. Furthermore, during increases in sound intensity by 5dB (or even less), the loss (up to 50%) of ribbons and neural terminal could be compensated by doubling the discharge rate or the number of firing neurons; this doubling in discharge is explained by the steep increase in cochlea discharge rate (Kujawa and Liberman, 2009).

There are two subtypes of afferent neurons, classified as low threshold and high threshold subtypes (Liberman, 1978). Each subtype is classified based on the spontaneous discharge rate (SDR), where a low threshold has higher SDR and the opposite for high threshold subtype (Liberman, 1978). So, if the noise mainly

impacts the high threshold subtype (which has low SDR), the sensitivity also would not change (Lin et al., 2011). However, there is no study showing the difference of the noise impact on any of these subtypes. As explained above, the ribbon synapse damage does not affect the sensitivity threshold, but based on our observation it affected the temporal processing.

4.6 Limitations and Future Directions

The first limitation of this study is that we did not track the ribbon loss longer than 5 weeks after the exposure. Longer term tracking would further detail the time course for ribbon loss and repair. However, further recovery of ribbon count is unlikely because the percentage of ribbon loss is roughly corresponding to SGN loss identified 2 years later in Lin et al.'s (2011) study. Secondly, the nature of damage to ribbon synapses has remained unclear; therefore, many issues remain to be explored. As mentioned above, immunostaining against CtBP2 has limitations, as staining only against CtBP2 is not enough to represent the fate of ribbon synapses after the noise exposure; therefore, staining against A-domain and other ribbon proteins is needed in order to detail the ribbon damage.

In the present study, I used guinea pigs because they are commonly used in auditory research; also they have much larger cochlea than mice. On the other hand, the C57BL/6J mice were used because they are available to use at this time, and the study on these mice is preliminary to see the impact of low level noise on temporal processing. So, the third limitation is the lack of morphology data from

our C57 mice, which might be different from guinea pigs, as reported from a CBA/CaJ mice study (Kujawa & Liberman, 2009). However, while our study confirmed variation between guinea pigs and CBA mice, the variation is unknown between C57 mice and CBA mice or guinea pigs. Specifically, variations in vulnerability to noise exist between two different strains of mice (Yoshida et al., 2000).

Fourthly, I did not record from an age-matched control group of mice, in order to exclude the possibility of aging effect on temporal processing, especially since aging hearing loss is common in this strain of mice.

Finally, the effect of long-term noise exposure should be evaluated, because it would simulate daily noise exposure in our life.

4.7 The Conclusion

Quick and massive loss of ribbons in guinea pig cochlea was seen shortly after a brief exposure to noise that does not cause permanent threshold shift, similar to what was reported in mice (Kujawa & Liberman, 2009). Unlike what was reported in mice (Kujawa & Liberman, 2009), the initial loss of ribbon in guinea pig cochlea was largely recovered within 5 wks after the noise exposure, with dislocation and size increase. The loss of ribbons largely impacted the temporal processing; this is shown by: (1) larger reduction in the amplitude of the second clicks response at shorter ISI's (both in CAP and ABR from guinea pigs and in

ABR from mice); and (2) increased CAP latency of the second click responses. Despite the full recovery in threshold, the CAP amplitude and temporal processing were not fully recovered 5 weeks after the noise; this is consistent with the remaining loss of ribbons at this time.

This study has shown that a cross-species variation in regard to noise impact on ribbon synapses does exist, and that ribbon synapses in guinea pigs are repaired and recovered after noise exposure. This variation between guinea pigs and mice is consistent with what was reported about the variation in SGN loss (Kujawa & Liberman, 2009; Lin et al., 2011). Finally, our test for temporal processing succeeded to be a sensitive test for temporal processing deterioration, and could give an idea about ribbon recovery after the noise damage.

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