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Relationship between auditory thresholds, central spontaneous activity and hair cell loss after acoustic trauma

W.H.A.M. Mulders^{*,1}, D. Ding², R. Salvi², and D. Robertson¹

¹The Auditory Laboratory, School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley WA 6009, Australia

²Center for Hearing and Deafness, University at Buffalo, Buffalo, NY 14214, USA

Abstract

Acoustic trauma caused by exposure to a very loud sound increases spontaneous activity in central auditory structures such as the inferior colliculus. This hyperactivity has been suggested as a neural substrate for tinnitus, a phantom hearing sensation. In previous studies we have described a tentative link between the frequency region of hearing impairment and the corresponding tonotopic regions in the inferior colliculus showing hyperactivity. In this study we further investigated the relationship between cochlear compound action potential threshold loss, cochlear outer and inner hair cell loss and central hyperactivity in inferior colliculus of guinea pigs. Two weeks after a 10 kHz pure tone acoustic trauma, a tight relationship was demonstrated between the frequency region of compound action potential threshold loss and frequency regions in the inferior colliculus showing hyperactivity. Extending the duration of the acoustic trauma from 1 to 2 h did not result in significant increases in final cochlear threshold loss, but did result in a further increase of spontaneous firing rates in the inferior colliculus. Interestingly, hair cell loss was not present in the frequency regions where elevated cochlear thresholds and central hyperactivity were measured, suggesting that subtle changes in hair cell or primary afferent neural function are sufficient for central hyperactivity to be triggered and maintained.

Keywords

tinnitus; inferior colliculus; guinea pig; cochleogram; compound action potential

Introduction

Exposure to intense sounds has long been known to cause hearing loss and tinnitus (Atherley et al., 1968; Loeb and Smith, 1967). While noise-induced hearing loss is often associated with cochlear pathologies such as hair cell loss or stereocilia damage (Liberman and Beil, 1979; Salvi et al., 1979); the structural changes associated with tinnitus are still poorly understood. What is clear is that loss of peripheral auditory thresholds caused by over-exposure to loud sound has been shown to result in increased spontaneous activity at multiple levels of the central auditory pathway, such as the cochlear nucleus, inferior colliculus and cortex (Bauer et al., 2008; Brozoski et al., 2002; Dong et al., 2010; Kaltenbach and Afman, 2000; Kaltenbach et al., 2004; Komiya and Eggermont, 2000; Ma et al., 2006; Mulders and Robertson, 2009; Seki and Eggermont, 2003). Spontaneous

hyperactivity has been suggested as a substrate for tinnitus, i.e. the perception of sound without an external stimulus (Bauer et al., 2008; Brozoski et al., 2002; Kaltenbach et al., 2004; Vio and Holme, 2005).

Previously we have shown that spontaneous hyperactivity in the central nucleus of the inferior colliculus (CNIC) after recovery from acoustic trauma is restricted to particular frequencies (Dong et al., 2010; Mulders and Robertson, 2009), specifically frequencies associated with peripheral threshold loss (Mulders and Robertson, 2009). This has also been suggested previously by others using cluster surface recordings in cochlear nucleus (Kaltenbach and Afman, 2000; Kaltenbach et al., 2000) and single neuron recordings in auditory cortex (Seki and Eggermont, 2003).

However, in our previous study (Mulders and Robertson, 2009) the number of neurons recorded was not large enough to investigate the fine-grain distribution of hyperactive neurons along the frequency axis and allow a detailed comparison with the peripheral threshold loss. In addition, the relationship between hair cell loss and spontaneous hyperactivity remains unclear. Some have reported hyperactivity in CNIC or dorsal cochlear nucleus (DCN) in the (1) absence of hair cell loss, (2) loss of outer hair cells with retention of inner hair cells, and (3) extensive loss of both inner and outer hair cell (Bauer et al., 2008; Kaltenbach et al., 2002). In an effort to better understand the functional and structural changes in the cochlea that give rise to hyperactivity, we obtained a larger sample of single neuron recordings along the frequency axis of the CNIC after acoustic trauma and examined in detail the frequency relationship between hyperactivity and peripheral threshold loss and between hyperactivity and tonotopic location of hair cell loss in the cochlea. We used two acoustic trauma paradigms of different duration to determine if the degree of peripheral hearing loss or cochlear hair cell loss was correlated with the pattern of hyperactivity in the CNIC.

Materials and Methods

Animals

Twelve adult pigmented guinea pigs of either sex, weighing between 260 and 315 g at the time of acoustic trauma, were used. The experimental protocols conformed to the Code of Practice of the National Health and Medical Research Council of Australia, and were approved by the Animal Ethics Committee of The University of Western Australia.

Initial surgery for acoustic trauma and sham controls

Following a subcutaneous injection of 0.1 ml atropine sulphate (0.6 mg/ml), animals received an intraperitoneal injection of Diazepam (5 mg/kg), followed 20 minutes later by an intramuscular injection of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; 1 ml/kg). When deep anaesthesia was obtained as determined by the absence of the foot withdrawal reflex, animals were placed on a heating blanket in a soundproof room and mounted in hollow ear bars. A small opening was made in the bulla in order to place an insulated silver wire on the round window. A compound action potential (CAP) audiogram (Johnstone et al., 1979) for the frequency range 4–24 kHz was recorded to assess the animals' cochlear sensitivity. All sound stimuli were presented in a closed sound system through a 1/2" condenser microphone driven in reverse as a speaker (Bruel and Kjaer, type 4134). Pure tone stimuli were synthesized by a computer equipped with a DIGI 96 soundcard connected to an analog/digital interface (ADI-9 DS, RME Intelligent Audio Solution). Sample rate was 96 kHz. The interface was driven by a custom-made computer program (Neurosound, MI Lloyd), which was also used to collect single neuron data during the final experiments. CAP signals were amplified, filtered (100 Hz–3 kHz bandpass) and

recorded with a second data acquisition system (Powerlab 4SP, AD Instruments). When cochlear sensitivity was within the normal range, the animal was randomly assigned to one of the following groups. Group 1 served as sham controls (n=4). These animals received no further treatment and the wounds were sutured and they were allowed to recover before the final experiment. In group 2 and 3 the contralateral ear was blocked with plasticine and the animal was subjected to an acoustic trauma. Group 2 animals were exposed to a continuous loud tone for 1 h (10 kHz, 124 dB SPL; n=4) and the group 3 animals (n=4) were exposed to the same continuous loud tone for 2 h. After the acoustic trauma another CAP audiogram was measured, the wound was sutured and buprenorphin (0.05 mg/kg subcutaneously) was given post-operatively as analgesic. Survival time in all animals varied between 13 and 16 days.

Surgery for final experiments

Animals received a subcutaneous injection with 0.1 ml atropine followed by an intraperitoneal injection of Nembutal (pentobarbitone sodium, 30 mg/kg) and a 0.15 ml intramuscular injection of Hypnorm. Maintenance anaesthesia regime consisted of full Hypnorm doses every h and half doses of Nembutal every 2 h. Animals were placed on a heating blanket in a sound proof room and artificially ventilated on carbogen (95% O₂ and 5% CO₂). Paralysis was induced with 0.1 ml pancuronium bromide (2 mg/ml intramuscularly). The electrocardiogram was continuously monitored and heart rate never increased over pre-paralysis levels at any stage of the experiments. After the animals were mounted in hollow ear bars, the left and right cochleae were exposed and CAP audiograms were recorded on both sides with a silver wire placed on the round window as for the initial experiment.

Inferior colliculus recording

To obtain extracellular single neuron recordings in the central nucleus of the inferior colliculus (CNIC) a small craniotomy overlying the visual cortex was performed and a glass-insulated tungsten microelectrode (Merrill and Ainsworth, 1972) was advanced along the dorso-ventral axis through the cortex into the contralateral CNIC (from the exposed cochlea) using a stepping motor microdrive. Electrode placement in the CNIC (about 2.5 to 3mm ventral to the cortical surface) was indicated by the presence of strong sound-driven activity with a short latency and a systematic progression from low to high characteristic frequencies (CF) with increasing depth. The craniotomy was covered with 5% agar in saline to improve mechanical stability. When a single unit was isolated its CF and threshold at CF were determined audio-visually and depth from the cortical surface was recorded. The spontaneous firing rate was measured for a period of 10 s.

Preparation of cochleograms

At the end of the experiment animals from group 2 and 3 received an injection with 0.2 ml Lethabarb (sodium pentobarbitone 325 mg/ml; VIRBAC) and were transcardially perfused by saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) after which both left and right cochleae were removed. The isolated cochleae were put in 4% paraformaldehyde in 0.1 M phosphate buffer, the round and oval windows were perforated, and a small hole was made in the apex. Fixative was then gently perfused through each cochlea for approximately 5 minutes after which the cochleae were stored in the same fixative at 4°C until further processing.

As described previously, cochleas were microdissected to remove the organ of Corti (Ding et al., 2003b; Zheng et al., 1999). The tissue was stained with Ehrlich's hematoxylin solution, mounted in glycerin on glass slides and viewed with a Zeiss microscope (Zeiss Standard, 400× magnification) using differential interference contrast optics. Hair cells were

counted as present if the cell body and cuticular plate were intact, and as missing if a phalangeal scar was present. Counts made over successive 0.24 mm segments along the entire length of the cochlea and entered into a custom software program. For each animal, percent hair cell loss was computed as a function of percent distance from the apex using lab norms from 6 young, healthy albino guinea pigs (Ding et al., 2003a). Using lab norms, cochleograms showing the percent hair cell loss as a function of percent distance from the apex were constructed for each animal. The results from each animal in the same group were averaged across animals to obtain a mean cochleogram. Cochlear position was related to frequency using a cochlear frequency-place map (Tsuji and Liberman, 1997).

Data analysis

Loss of peripheral sensitivity can affect the CF based on the lowest threshold of tuning curves in the damaged region; therefore in every animal we converted the recorded unit's electrode depth into the CNIC (from cortical surface) into frequency (see also (Mulders and Robertson, 2009) during post-experimental analysis. For this purpose audiovisually estimated CF versus depth was plotted for all neurons in the regions without affected thresholds and a curve was fitted to these data using a second-order polynomial function. The formula of the fitted curve was then used to transform the depth value of the units recorded into nominal CF. Figure 1 illustrates this CF adjustment process. Figure 1A shows that the relationship between depth (in μm) and audiovisually estimated CF can be well fitted using a polynomial function (in this animal the function was $0.000003136 \cdot \text{depth}^2 - 0.01457 \cdot \text{depth} + 15.3$). Figure 1 B shows the results from an animal with a substantial hearing loss between 10 and 24 kHz. In such an animal only neurons with a CF up to 8 kHz (where CAP was shown to be normal) (black circles) were used to determine the polynomial function ($\text{CF} = 0.000004871 \cdot \text{depth}^2 - 0.02941 \cdot \text{depth} + 45.71$). There is a definite tendency for neurons with CFs lying within the damaged region to have CFs lower than predicted, in agreement with the known CF shift caused by loss of the tuning curve tip (Cody and Johnstone, 1980). Figure 1C shows the effect of transforming audiovisual CF into a nominal CF. Nominal CF, estimated in the way described above, is used in all subsequent figures.

To identify statistically significant difference in spontaneous firing rates between groups as a function of frequency regions in the IC, the Kruskal-Wallis test and Dunn's multiple comparison post test were used. Statistical analysis of CAP threshold changes at each frequency was performed using a one-way ANOVA a Bonferroni multiple comparisons post test.

Results

Cochlear thresholds

Both 1 h and 2 h acoustic trauma regimes caused an immediate large elevation of ipsilateral CAP thresholds after the exposure at most frequencies (Fig. 2A,B). With both the 1 and 2 h exposure the elevation was significant at all frequencies 6 kHz and higher (Fig. 2A and B). Two weeks after acoustic trauma, ipsilateral (left) CAP thresholds recovered substantially after both durations of acoustic trauma showing a small remaining threshold loss (Fig. 2A, B and C). Mean threshold loss after recovery was significantly different from pre-surgery levels at 12, 20, 22 and 24 kHz after 1 h exposure (Fig. 2A) and at 12, 14, 20, 22 and 24 kHz after 2 h exposure (Fig. 2B). Although the CAP threshold changes were consistently larger for 2 h compared to 1 h exposures, the differences were not significant, probably as a result of the considerable inter-animal variability (Fig. 2C). For example threshold loss at 12 kHz varied between 15 and 30 dB and between 19 and 45 dB after the 1 h or 2 h exposure, respectively. CAP thresholds were also measured on the side contralateral (right cochlea) to

the acoustic trauma and were shown to be unaffected by left acoustic trauma, i.e., no statistically significant differences (Fig. 2A and B).

In sham control animals, after 2 weeks of recovery from surgery, CAP thresholds did not change significantly at any of the frequencies tested (Fig. 2D), demonstrating that the surgery alone did not significantly affect hearing thresholds. In addition, there was no significant difference between the initial CAP thresholds recorded from the left or right cochlea (Fig. 2D).

Spontaneous activity in IC

Mean spontaneous activity of all measured CNIC neurons was 1.6 ± 0.2 spikes/sec (ranging from 0 to 36.6 spikes/sec; $n=408$ neurons) in sham control animals, 4.7 ± 0.4 spikes/sec (ranging from 0 to 64.8 spikes/sec; $n=545$ neurons) after 1 h acoustic trauma and 6.3 ± 0.5 spikes/sec (ranging from 0 to 65.6 spikes/sec; $n=573$ neurons) after 2 h of acoustic trauma. Statistical comparison using the Kruskal-Wallis test and Dunn's multiple comparison post-test showed that the spontaneous firing rates of the two experimental groups were statistically significant different from shams ($p<0.001$) and that the 2 h exposure lead to statistically significantly higher spontaneous firing rates in CNIC compared to the 1 h exposure ($p<0.01$) (Fig. 3).

In order to determine whether the spontaneous firing rates were increased throughout the CNIC or were limited to specific frequency regions, neurons were grouped according to CF. As described in the Methods, in every animal, we converted the depth in the CNIC into nominal frequency (see also (Mulders and Robertson, 2009)). Figure 4 shows the distribution of spontaneous activity of CNIC neurons according to nominal CF. The spontaneous activity in the exposed groups increased with CF whereas in the sham animals the spontaneous firing rate was low at all CFs. In the exposed animals the spontaneous firing rate was clearly less at CFs <9 kHz compared to CFs >9 kHz. Statistical analysis showed that at frequencies >12 kHz the spontaneous activity after both 1 h and 2 h exposures was significantly increased compared to the shams with the exception of CF >21 kHz in the 1 h exposure group, which may be due to the large variance and low number of neurons recorded in this frequency region. In addition, the spontaneous rates in the 2 h group tended to be higher than those in the 1 h group; however, there was only a statistically significant difference between the 1 h and 2 h exposed groups in the >12 – 15 kHz range.

The relationship between the distribution of spontaneous activity in the CNIC and cochlear CAP thresholds was investigated by plotting spontaneous activity as a function of CF. Figure 5A shows data from all individual neurons from the 2 hr acoustic trauma group. The data suggests a tendency to higher spontaneous firing rates in regions of substantial CAP threshold loss. Figure 5B shows the same single neuron data in 2 kHz CF bins. The shape of the distribution resembles that of the CAP threshold loss shown in figure 5A. In order to further illustrate this relationship we used a smoothing procedure of the single neuron data. This involved ordering neurons according to CF and then averaging spontaneous firing rates for a predetermined number of neurons above, at and below each neuron (running average). Figure 5C and 5D show the effect of this procedure using 11 or 31 neurons in the running average, respectively. It is apparent that the distribution of spontaneous firing rates is qualitatively similar to that shown in B. Furthermore there is a clear correlation between CAP threshold loss and distribution of spontaneous firing rates. Increasing the number of neurons in the running average from 11 to 31 greatly reduced the fluctuations in the data but did not produce any obvious change in the distribution of data according to CF.

Figure 6 shows the comparison of a 31 point running average in both the 1 h and 2 h acoustic trauma groups with both the temporary and permanent CAP threshold loss. A tight

correlation between the level of CAP threshold loss after recovery and the level of spontaneous activity recorded in the CNIC was observed for both the 1 h (Fig. 6C) and 2 h (Fig. 6D) exposed groups. The only minor exception to this tight correlation was seen above 21 kHz in the 1 h group, which may be due to the low number of neurons at the higher frequencies. The figure also demonstrates a lack of correlation of the spontaneous firing rates with the acute temporary threshold loss (Fig. 6A and B), suggesting the initial sensory deprivation is not triggering the spontaneous firing rate increase.

Cochleograms showed loss of inner and outer hair cells after both the 1 h and 2 h exposure (Fig. 7A and B). Inner hair and outer hair cell loss was restricted almost exclusively to the high frequency regions of the cochlea. After the 1 h acoustic trauma (Fig. 7A) inner hair cell loss started at the 25 kHz region and then slowly progressed to an approximate 40% loss at 43 kHz after which is quickly reached a 90% loss at 48 kHz. The outer hair cell loss was very similar in this group at the high frequencies. After the 2 h acoustic trauma, inner hair cell counts at high frequencies revealed a bimodal pattern (Fig. 7B). The cochleogram showed a gradual loss starting at 22 kHz peaking (20%) at 26 kHz and dropping off to 0% loss at 29 kHz. Then at 40 kHz hair cell loss became apparent again climbing steeply to about 50% loss at 50 kHz. The pattern of outer hair cell loss paralleled the inner hair cell loss at high frequencies but outer hair cell loss was greater than the inner hair cell loss.

To allow comparison of the hair cell loss with cochlear threshold loss, in Figure 7, CAP threshold loss was also plotted (note: CAP thresholds were only measured up to 24 kHz). No direct relationship is apparent between CAP threshold loss and hair cell loss. Indeed, CAP threshold losses of 20–30 dB were present between 10–20 kHz, yet there was little or no evidence of hair cell loss at these frequencies on the tonotopic map.

Discussion

Noise-Induced Threshold Loss

In agreement with previous noise-exposure studies, our acoustic trauma paradigms resulted in an initially very large threshold loss in the cochlea followed by a smaller persistent loss after two weeks recovery (Dong et al., 2009; Mulders and Robertson, 2009; Norena and Eggermont, 2005; Salvi et al., 1979; Seki and Eggermont, 2003). This persistent loss of peripheral sensitivity was present slightly above the exposure frequency which is thought to be due to the basal-ward spread of damage to inner and outer hair cells on the basilar membrane, away from the exposure frequency (Cody and Robertson, 1983; Robertson and Johnstone, 1980). This phenomenon arises from the nonlinear basilar membrane mechanics which result in larger amplitudes basal to the characteristic frequency peak when using high intensity sounds (Cody and Johnstone, 1981; Sellick et al., 1982).

In addition, our data also showed a persistent CAP threshold loss at much higher frequencies (20 to 24 kHz), a phenomenon also observed in previous studies (Dong et al., 2010; Mulders et al., 2010; Wang et al., 2002). The possible reasons for this more extended high frequency threshold loss are unclear. It could be a result of basalward shifts in the peak of vibration at high intensities. Another contributing factor could be the presence of harmonic distortion, which in our sound system was measured to be 20 dB or more below that of the primary tone, and therefore unlikely to cause permanent loss of peripheral sensitivity in guinea pig (Cody and Robertson, 1983), although there have been no systematic studies of the vulnerability of guinea pigs to high frequency (20–24 kHz) acoustic trauma. In addition, the higher frequency regions are likely to be more susceptible to acoustic trauma due to lesser amounts of protective antioxidant enzymes (Sha et al., 2001). The issue of unexpected damage in the extreme high frequency end of the cochlea has been extensively reviewed by Wang et al. (Wang et al., 2002).

Extending the duration of the acoustic trauma from 1 to 2 h resulted in similar amounts of temporary CAP loss (Fig 2A–B), but 5–10 dB greater persistent CAP losses between 12–20 kHz (Figure 2C). However, the magnitudes of the differences in persistent CAP threshold loss were not statistically significant (Fig. 2C). While it is conceivable that the 2-h exposure caused more cochlear threshold loss above the highest frequency that we measured (24 kHz) the differences are unlikely to be very great since the magnitude of the hair cell lesion from the 2-h exposure was not much different from the 1-h exposure.

Noise-Induced Hyperactivity

Both the 1 h and 2 h acoustic exposures resulted in a significant rise in spontaneous firing rates in CNIC neurons compared to neurons in sham animals, in agreement with previously published data from our laboratory and others (Bauer et al., 2008; Dong et al., 2009; Dong et al., 2010; Ma et al., 2006; Mulders and Robertson, 2009; Mulders et al., 2010). Increases in spontaneous firing rates after acoustic trauma have also been demonstrated in other parts of the auditory pathways, such as dorsal cochlear nucleus and auditory cortex (Brozoski et al., 2002; Kaltenbach et al., 2000; Norena and Eggermont, 2003; Seki and Eggermont, 2003). The available evidence indicates that primary afferent spontaneous activity does not show long-term increases in acoustic trauma models (Liberman and Dodds, 1984), indicating that the phenomenon of central hyperactivity involves central plasticity. In addition, confirming previous observations (Dong et al., 2009; Dong et al., 2010; Mulders and Robertson, 2009; Mulders et al., 2010) the rise in spontaneous firing rates was not present in all frequency regions but was confined to neurons with a CF > 12 kHz, i.e., near or above the exposure frequencies.

Persistent Threshold Loss and Hyperactivity

Plotting the running average of spontaneous firing rates against CF together with cochlear threshold loss against frequency revealed a tight correlation between peripheral threshold loss and spontaneous firing rate. Extending the duration of acoustic trauma from 1 to 2 h significantly increased the level of spontaneous firing rates in the CNIC (Figure 3); the mean spontaneous rate (irrespective of CF) in the 2 h group was significantly higher than that in the 1 h group and the mean spontaneous rates in exposure groups were significantly higher than the rate in the sham control group.

The effects of the exposure were frequency specific; both the 1 h and 2 h exposures caused the spontaneous rates to increase in neurons with CF > 12 kHz. While the spontaneous rates in the 2 h group were consistently higher than those in the 1-h group, the only difference that was statistically significant between the two groups was among neurons with CF between 12 and 15 kHz. It should be noted that the CAP threshold losses in this region (12–14 kHz) were 9–12 dB greater in the 2 h versus the 1 h exposure group. These results suggest that even small changes in cochlear sensitivity can have a significant impact on spontaneous rates in the auditory brain.

We observed a strong correlation the between loss of peripheral sensitivity and the increase in spontaneous firing rate in the CNIC as suggested by previous studies (Dong et al., 2009; Kaltenbach and Afman, 2000; Ma et al., 2006; Mulders and Robertson, 2009). However, in these earlier studies the evaluation was hindered by the fact that either surface recordings were used or not enough neurons were sampled per frequency region. Taken together, our results show that spontaneous firing rates in CNIC are in direct proportion to the degree of permanent cochlear hearing loss (Figure 6A–B). Further experiments are needed to elucidate whether the relationship between peripheral threshold loss and frequency regions of hyperactivity remains when the locus of peripheral threshold loss shifts to even lower frequencies. Finally, it is important to note that the CAP threshold losses measured

immediately after the noise exposure extended down into the 6–8 kHz region. While we did not observe a persistent CAP threshold loss or significant increase in spontaneous activity below 9 kHz, the spontaneous rates in 6–9 kHz regions were marginally higher in both of exposed groups than in the sham group.

Spontaneous Hyperactivity and Tinnitus Pitch

The striking relationship between the distribution of peripheral loss of sensitivity and frequency region of hyperactivity demonstrated by these results is interesting in the context of psychophysical data and models of tinnitus pitch derived from physiological studies. When temporary threshold shift is induced with pure tone, subjects match the pitch of their tinnitus to a tone located above the frequency of maximum hearing loss sometimes by as much as a half octave (Loeb and Smith, 1967). In contrast, when temporary hearing loss is induced by an octave or 1/3 octave band of noise, the pitch of the tinnitus is located below the maximum hearing loss (Atherley et al., 1968; Loeb and Smith, 1967). A somewhat different pattern has emerged from human studies of permanent hearing loss. Some report that the tinnitus frequency matches the edge of a sloping high-frequency hearing loss (Moore et al., 2010). On the other hand, when listeners were asked to numerically rate the contribution of individual frequencies to their tinnitus percept, the “internal tinnitus spectra” covered a broad frequency range resembling the hearing loss (Norena et al., 2002). According to these data, the tinnitus sensation is perceived as a high-frequency noise with tonal qualities. If we assume that tinnitus arises from spontaneous hyperactivity, then our broad profile of spontaneous hyperactivity should be perceived as a high pitched noise with 12 kHz tonal properties. One factor that needs to be considered when interpreting these results is that the data are from the CNIC, not the cortex where tinnitus perception presumably takes place. However, in contrast to the CNIC (Izquierdo et al., 2008), the auditory cortex is known to undergo tonotopic map reorganization (Robertson and Irvine, 1989), whereby the denervated cortical region is occupied by adjacent frequency regions. This means that the hyperactivity measured in the CNIC could be shifted into other frequency regions depending on the level of reorganization in the cortex.

The relationship between cochlear threshold changes, central hyperactivity and the spatial pattern of hair cell degeneration was complex. First, in both exposure groups, there was no substantial hair cell loss in cochlear regions corresponding to most of the frequencies at which measured cochlear thresholds were elevated, and where there was substantial spontaneous hyperactivity in the CNIC. This finding is important because it strongly suggests that the triggering and the maintenance of central hyperactivity, while closely related to peripheral threshold changes, does not require degeneration of either outer or inner hair cells in the affected frequency regions. It is possible that more subtle alterations in hair cell ultrastructure may be involved. For example, in agreement with the pattern of threshold changes and hyperactivity it has been previously demonstrated that damage to outer hair cells consisting of fusion and collapse of stereocilia occurs somewhat basalward of the exposure frequency location (Cody and Robertson, 1983; Robertson et al., 1980). In addition, diffuse neural changes, either to the Type I (inner hair cell) or Type II (outer hair cell) afferent innervation cannot be excluded (Kaltenbach et al., 2002; Kujawa and Liberman, 2009; Liberman and Dodds, 1984; Tsuprun et al., 2003).

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Figure 1.

Illustration of effects of conversion from audiovisually estimated CF into nominal CF. A: scatterplot of the relationship between depth from cortical surface and audiovisual CF of all neurons from one control animal and the fitted polynomial function. B: scatterplot of relationship between depth from cortical surface and audiovisual CF of all neurons from a 2 week acoustic trauma animal and the fitted polynomial function based on neurons with an audiovisual CF in the normal hearing regions (according to CAP). C: illustration of the effect of transforming audiovisual CF into nominal CF. of the animal shown in B. Line indicates 1:1 ratio.

Figure 2.

A,B: CAP thresholds at different frequencies recorded from the left cochlea before acoustic trauma (black circles), immediately after acoustic trauma (open triangles) and after a 2 week recovery period after acoustic trauma (black triangles), as well as from the right cochlea after recovery (open circles). A, 1 h acoustic trauma. B, 2 h acoustic trauma. C: CAP threshold losses recorded from the left cochlea at different frequencies two weeks after 1 h (black circles) and 2 h (open circles) of acoustic trauma. D: CAP thresholds at different frequencies recorded from the left cochlea of sham animals during initial surgery (black circles) and after a 2 week recovery period (black triangles), as well as from the right cochlea after recovery (open circles). Data points $\pm$ SEM. $n=4$ for all groups. * $p<0.05$; ** $p<0.01$; # $p<0.001$ statistical significance as compared to before trauma data. Grey line in A–C indicates exposure frequency.

Figure 3. Mean spontaneous firing rate of all CNIC neurons recorded in each group. Asterisks without bar indicate significance from shams. ** $p < 0.01$; *** $p < 0.001$.

Figure 4.

Mean spontaneous activity of single neurons in different frequency regions in the CNIC in sham animals (grey bars) and animals after 2 weeks recovery from 1 (white bars) or 2 h acoustic trauma (black bars). The number of recorded neurons in each frequency region is indicated above the bars. Bars show mean spontaneous firing rates $\pm$ SEM. * $P < 0.05$; ** $p < 0.01$; # $p < 0.001$.

Figure 5.

Illustration of relationship between CF and spontaneous firing rates of CNIC neurons in all 2 hr acoustic trauma animals and the CAP threshold loss at different frequencies. A: scatterplot of individual neurons and CAP threshold loss (mean $\pm$ SEM). B: Mean spontaneous activity of single neurons in different frequency regions (2 kHz bins) in CNIC. Bars show mean spontaneous firing rates $\pm$ SEM. C and D: 11 (C) and 31 (D) point running average of spontaneous firing rate and CAP threshold loss (mean $\pm$ SEM).

Figure 6.

Relationship between the distribution of spontaneous firing rates of CNIC neurons and CAP threshold loss according to frequency. A, 1 h acoustic trauma group, acute threshold loss. B, 2 h acoustic trauma group, acute threshold loss. C, 1 h acoustic trauma group, permanent threshold loss. B, 2 h acoustic trauma group, permanent threshold loss. $n = 4$ in all panels. CAP threshold loss shown $\pm$ SEM. Spontaneous firing rate shown as a 28 point running average. Grey line indicates exposure frequency.

Figure 7. cochleograms showing missing inner (dark line) and outer hair cells (dotted, medium and thin black lines) along the tonotopic axis of the cochlea after 1 h (A) and 2 h (B) of acoustic trauma in combination with the mean CAP threshold loss in both groups. All data $n=4$. Grey line indicates exposure frequency.