Changes in spontaneous neural activity in the dorsal cochlear nucleus following exposure to intense sound: relation to threshold shift

James A. Kaltenbach a,*, Donald A. Godfrey b, John B. Neumann a, Devin L. McCaslin a, Chad E. Afman a, Jinsheng Zhang a

a Department of Otolaryngology, SE-UHC, Wayne State University, Detroit, MI 48201, USA
b Department of Otolaryngology, Medical College of Ohio, Toledo, OH 43699-0008, USA

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Abstract

Previous studies have shown that the dorsal cochlear nucleus exhibits increased spontaneous activity after exposure to intense sound. Such increases were apparent 1–2 months after the exposure and were generally proportional to the shift in response thresholds induced by the same exposure. The purpose of the present study was to determine whether this sound-induced increase in spontaneous activity is an early event which can be observed shortly after exposure. As in previous studies, anesthetized hamsters ranging in postnatal age from 60–70 days were exposed to a 10-kHz tone at levels between 125 and 130 dB SPL for a period of 4 h. Control animals were similarly anesthetized but were not exposed to the intense tone. Exposed animals were examined in two groups, one at 30 days after exposure, the other at 2 days after exposure. Time of exposure was adjusted so that all animals were between 90 and 100 days of age when spontaneous activity was studied electrophysiologically. The results showed that the increases in spontaneous activity, which were evident at 30 days after exposure, were not observed in animals studied 2 days after exposure. This result contrasted with the effect of the intense tone exposure on neural response thresholds. That is, the shifts in response thresholds seen 2 days after exposure were similar to those observed in animals studied 30 days after exposure. These results indicate that changes in spontaneous activity reflect a more slowly developing phenomenon and occur secondarily after induction of threshold shift.

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1. Introduction

When the cochlea is injured or overstimulated by intense sound, central auditory structures usually show evidence of this injury by responding more weakly to sound. This loss of peripheral sensitivity is reflected at many levels of the central auditory system from the cochlear nuclei to the auditory cortex (see Syka, 1989, for review). However, exposure to intense sound may also trigger secondary alterations which do not correspond to those observed at the peripheral level. Examples include reorganizations of the tonotopic map (Har-
those seen in the DCN of unexposed hamsters. This increase was generally proportional to the average change in neural response thresholds, with maximal activity occurring at or near the portion of the DCN representing the frequency of the exposure tone and displaying the severest threshold shifts.

This sound-induced hyperactivity is of considerable interest because of its relevance to tinnitus, an auditory disorder which is thought by some investigators to involve disturbances of spontaneous activity at central levels of the auditory pathway (Jastreboff and Sasaki, 1986; Chen and Jastreboff, 1995; Kitahara et al., 1995; Kaltenbach and McCaslin, 1996a; Wallhauser-Franke et al., 1996). The importance of central components of tinnitus is suggested by clinical reports showing that tinnitus often persists after surgical resection of the auditory nerve (Dandy, 1941; House and Brackman, 1981; Soussi and Otto, 1994). The notion that tinnitus might involve disturbances in spontaneous activity is suggested by studies showing that animals treated with sodium salicylate experience tinnitus-like percepts (Jastreboff et al., 1988, 1989) and show evidence of altered spontaneous activity at several levels of the auditory pathway (Evans et al., 1981; Evans and Borrerve, 1982; Jastreboff and Sasaki, 1986; Wallhauser-Franke et al., 1996).

Intense sound exposure is the most commonly cited cause of chronic tinnitus (Axelsson and Barrenas, 1992; Axelsson and Ringdahl, 1989; Coles, 1995; see also McFadden, 1982). However, previous studies indicate that intense noise exposure causes a chronic decrease in the spontaneous activity of auditory nerve fibers (Liberman and Kiang, 1978; Liberman and Dodds, 1984). One study reported a slight increase in spontaneous activity of auditory nerve fibers after intense noise exposure, and this increase was not found to be statistically significant though much weaker than that seen in the hamster. While other studies have shown that the activity of some auditory nerve fibers may increase after acute sound exposure (Lonsbury-Martin and Martin, 1981), such increases were found to be short lived, disappearing within a few seconds or minutes following stimulation. Thus, chronic increases in spontaneous activity in the DCN following intense tone exposure do not have any known parallels at the peripheral level, and it is therefore likely that they originate centrally.

The present study focuses on the onset of this hyperactivity following intense sound exposure. We sought to determine whether the increase in spontaneous activity seen in the DCN one month after intense sound exposure is also seen after only two days postexposure. The motive for this study was to establish whether shifts in spontaneous activity have an early onset of occurrence similar to the shifts in response threshold or, instead, follow secondarily after induction of threshold shift. As in previous studies, measures were performed along a systematic series of tonotopic coordinates on the DCN surface to pinpoint the loci of maximal impact of tone exposure. The results indicate that changes in spontaneous activity do not occur at the same time as shifts in response threshold, suggesting that the increases in spontaneous activity are a secondary disturbance involving central mechanisms.

2. Methods

2.1. Animal subjects

Animals were adult Syrian golden hamsters obtained from Charles River Laboratories. These were housed in a university animal facility in accordance with NIH guidelines on the care and use of animals. All animals were between 60 and 70 days of age on the day of arrival.

2.2. Sound exposures

Sound exposures were carried out in a group of 10 animals within 1–4 days of their arrival. Another group of 10 animals was exposed between the third and fourth week after their arrival so that the effects of exposure could be studied in animals of similar age but at 2 different postexposure recovery times, one at 2 days and one at 30 days. Exposures were performed inside a sound attenuation booth (IAC) using a Beyer DT 48 transducer. The output side of the transducer was equipped with a conical rubber funnel. A hollow 1-cm tube cut from a plastic 1-cc syringe was inserted into the orifice of the conical funnel and served as an extension tube which provided a means of focusing sound into a beam slightly larger than the diameter of the concha. The exposure signal was a continuous 10-kHz sinusoid whose level was initially measured by placing the end of a probe tube microphone (Etymotic ER7C) at the tip of the extension tube with no animal present. The output of the microphone was fed to a Rapid Systems spectrum analyzer, and the amplitude of the exposure tone was adjusted until the level of the fundamental measured 127 dB SPL at the tip of the extension tube. Harmonics typically varied in level between 40 and 60 dB below the level of the fundamental.

Following this initial measurement, the exposure signal was turned off and the microphone was removed. For each exposure an animal was anesthetized by intramuscular injection of a mixture of ketamine (113 mg/kg) and xylazine (17 mg/kg) and placed on a heating pad inside the sound attenuation booth. A rectal probe sensor was used to regulate the animal’s temperature throughout the exposure period. The animal was then positioned for sound exposure. The speaker was ori-
ent so that the extension tube was inserted into the left pinna and the tip of the tube was within 1–2 mm of the concha. The exposure tone was then switched on and maintained without interruption for a period of 4 h. The open field level of the tone was continuously monitored throughout the exposure period by positioning the probe tube microphone just above the animal’s head. The exposure was discontinued, and the animal was removed from the study if the exposure level changed by more than 3 dB over the 4-h period. Control animals were age-matched to the exposed animals and were similarly anesthetized for a period of 4 h but without the exposure sound.

2.3. Recordings of multiunit spontaneous activity

Experiments were performed in two postexposure recovery groups. The first group was examined 30 days after tone exposure, and the second group at 2 days after exposure. The electrophysiological procedures used in these experiments were similar to those described elsewhere (Kaltenbach and McCaslin, 1996a; Kaltenbach et al., 1992; Meleca et al., 1997) with minor modifications. Briefly, all experiments were performed by recording multiunit activity at the DCN surface using micropipette electrodes with tip impedances between 0.4 and 0.5 MΩ and filled with 0.3 M NaCl. The electrode was mounted on a hydraulic microdrive, then manually positioned just above the left DCN. Further movement of the electrode was controlled remotely by a Narashige XYZ micromanipulator. The electrode was lowered until the tip made contact with the DCN surface as indicated by a sudden emergence of neural activity. Neural potentials were amplified 1000× using a preamplifier with a bandpass setting of 300–10 000 Hz. The amplified signals consisted of complex waveforms representing the activity of a few neurons with overlapping action potentials (see Kaltenbach and McCaslin, 1996a). A level discriminator was adjusted to trigger on potentials exceeding −100 mV (after amplification). The resulting trigger pulses were then fed to a universal counter, and the number of pulse events was counted over a period of 90 s. These counts were then converted to spontaneous rates, expressed as the number of events per second. Such measures were obtained in 3 rows of 10–13 (30–39 total) recording sites along the tonotopic axis of the DCN, oriented parallel to the medio-lateral axis (Kaltenbach and Lazor, 1991). These sites were spaced approximately 100 µm apart and spanned most of the medio-lateral width of the DCN except the medial-most portion representing frequencies above 20 kHz as described previously (Kaltenbach and Lazor, 1991). Any animal showing an indication of surgically-induced changes in either the physiology or surface anatomy of the DCN was excluded from the sample.

2.4. Recordings of frequency-threshold curves

Recordings of frequency tuning properties were obtained at some of the same locations from which recordings of spontaneous activity were obtained. The methods for mapping these properties across the DCN surface were the same as those used in previous studies with minor modifications (Kaltenbach et al., 1992, 1996b; Kaltenbach and Lazor, 1991; Meleca et al., 1997). In exposed animals, only the sites in the most lateral portion of the DCN were mapped because multiunit response areas in more medial regions of the DCN did not retain normal tuning properties (Kaltenbach and McCaslin, 1996a). At each site, neural response areas were obtained and used to measure threshold at the characteristic frequency (CF), defined as the frequency of lowest threshold. The CFs at all such sites were then plotted on a drawing of the DCN and the locus of neurons tuned to 5 kHz was identified in each of the 3 rows of sites.

2.5. Data analysis

In each animal, spontaneous rates and CF thresholds were plotted vs. distance from the 5-kHz iso-frequency contour line on the DCN surface. The mean medio-lateral width of the DCN spanned by the recording sites (usually between 1.0 and 1.2 mm) from each animal was then divided into bins, each representing 0.1 mm of tissue. All spontaneous rates falling within a given bin were first pooled within a given animal to calculate a mean rate. The means from corresponding bins were then pooled and averaged across animals within the same group, yielding a mean for each bin for each animal group. Comparisons between groups were made by plotting the mean rates or thresholds ± 1 S.E.M. The effects of tone exposure were evaluated by computing the differences between mean spontaneous rates or CF thresholds in tone-exposed and control animals for each of the bins.

3. Results

All of the recordings in the present study were obtained directly from the DCN surface without penetrating subsurface structures. However, the surface was not the locus where spontaneous activity was maximal in the DCN. In selected animals after the present study was completed, we found that spontaneous rates increased to a maximum when the electrode was moved to an average position of 160 µm below the surface. Since the purpose of the present study was to compare activity at different positions along the medio-lateral (tonotopic) axis, recordings were restricted to the DCN surface where variability in spontaneous rates...
related to regional differences in cell density or to electrode-induced damage to synaptic structures could be minimized.

3.1. Thirty days postexposure

3.1.1. Spontaneous activity

Exposure to intense sound caused major increases in spontaneous activity in the DCN. This is shown in Fig. 1, which compares mean spontaneous rates in 10 exposed animals with mean rates in 6 age-matched controls. Mean rates were consistently higher in exposed animals across most of the tonotopic range of the DCN, reaching a peak at a position approximately midway between the medial and lateral extremities of the DCN. This peak was observed at a locus corresponding to the position which normally responds best to 10 kHz (see frequency scale below abscissa), the frequency of the exposure tone. At this position spontaneous rates averaged 78 events per second in exposed animals, approximately 5 times the average at the corresponding position in control animals (15 events per second).

3.1.2. Thresholds

The 30-day control values for response thresholds vs. distance along the medio-lateral (tonotopic) axis of the DCN are shown by the lower dashed curve in Fig. 2. Thresholds were distributed along a wide U-shaped function and were lowest in the middle region of the DCN and highest in the extreme medial and lateral regions. Corresponding thresholds measured in the 30-day postexposure group are shown by the upper dashed curve in this figure. As can be seen, there was an increase in thresholds relative to control values over much of the DCN, with maximal shifts of more than 60 dB occurring between 0.3 and 0.7 mm medial to the 5-kHz isofrequency contour, a position approximately centered on the 10-kHz isofrequency contour (see frequency axis below abscissa). Thresholds were near normal in the lateral (low frequency) portion of the DCN and shifted by 38 dB in the medial (high frequency) portion of the DCN.

3.2. Two days postexposure

3.2.1. Spontaneous activity

In contrast to the robust increases in spontaneous activity seen in the 30-day postexposure group, no such increases were observed in animals at 2 days after exposure. This is shown in Fig. 3 comparing mean spontaneous rates from the 6 animals studied 2 days postexposure with those recorded from 4 unexposed, age-matched controls. Here, it is apparent that in the exposed animals mean rates did not exceed 14 events per second. This value was lower than the maximal mean value of 34 events per second in controls; it also contrasts with the maximal value recorded 30 days after exposure, which averaged 78 events per second (see Fig. 1).
The difference between activity in acutely and chronically recovered exposed animals is more directly displayed in the graph of Fig. 4 plotting distributions of maximal spontaneous rates recorded across all animals in each of these animal groups. As can be seen, the acutely recovered animals displayed a range of maximal rates between 21 and 51 events per second. This only slightly overlapped that of chronically recovered animals: 43–160 events per second.

3.2.2. Thresholds

The 2-day postexposure control values for mean response thresholds vs. distance along the tonotopic axis of the DCN are shown by the lower solid curve in Fig. 2. Mean thresholds were close to those of the 30-day control curve (lower dashed curve), with lowest values occurring in the middle region of the DCN and highest in the medial and lateral extremities. In contrast, mean thresholds for the 2-day postexposure group were systematically shifted toward the middle region of the DCN as shown by the upper solid curve in Fig. 2. The amount and pattern of threshold shift seen at 2 days after exposure was thus similar to that seen in the 30-day postexposure group except in the lateralmost portion of the DCN.

4. Discussion

It is apparent from these data that increases in spontaneous activity seen at 30 days after exposure are not observed 2 days after exposure. This result contrasts with the changes in threshold shifts which were seen at both 2 and 30 days after exposure. In interpreting the differences in spontaneous activity observed at 2 and 30 days after exposure, it is important to consider the possibility that such differences might be more related to the different ages at which animals in the two groups were exposed to intense sound rather than to differences in postexposure recovery time. In the present study animals in the 2-day recovery group were exposed nearly a month after animals in the 30-day group. This experimental design was chosen so that animals in the two groups would be age-matched at the time of electrophysiological study, thus avoiding possible age-related changes in spontaneous activity, independent of tone-exposure. However, this approach would not have excluded effects resulting from age-related differences in the susceptibility to acoustic injury (see Saunders and Tilney, 1982). In the hamster, such differences have been observed for a period spanning the first two months of postnatal development (Bock and Saunders, 1977). In the present study we attempted to avoid the confounding effects of this developmental artifact by exposing our hamsters to sound after they reached 60–70 days of age. We also performed a pilot investigation similar to the one reported here except that the animals were exposed at the same age and thus were not age-matched at the time of electrophysiological study. As in the present study, spontaneous activity was found to be increased one month after exposure but not after 2–3 days (Kaltenbach and McCaslin, 1996b). Taken together, these considerations led us to interpret the increased activity at 30 days as being unrelated to age differences at the time of exposure.

These findings provide a new perspective on the re-

![Fig. 3. Mean multiunit spontaneous rates plotted vs. distance along the medio-lateral axis of the DCN for the 2-day experiment. Each point is the average of mean values ± S.E.M. measured across animals (n=6 for exposed group; n=4 for control group). In each graph the distance was measured relative to the 5-kHz contour line of the DCN as viewed from a dorsal perspective. Locations of corresponding frequencies are shown by the scale below the abscissa. All rates are based on an event counting interval of 90 s using a trigger level of −100 mV.](image1)

![Fig. 4. Comparison of maximal spontaneous rates in exposed animals studied at 2 and 30 days after exposure with those inagematched controls. Each point represents the maximum rate measured in a single animal.](image2)
lation between threshold shift and changes in spontaneous activity induced by sound exposure. Previously, we showed that shifts in spontaneous activity following tone exposure were approximately proportional to the amounts of neural threshold shift when animals were studied 1–2 months after exposure (Kaltenbach and McCaslin, 1996a). This raised the possibility that the two changes were linked in such a way that spontaneous activity shifted as a direct consequence of reduced sensitivity. The results of the present study indicate that the relationship between increased activity and threshold shift is not a direct one. Although both thresholds and spontaneous activity were shifted at 30 days after exposure, no such relation was apparent when animals were studied at 2 days after exposure. Spontaneous activity was not increased at this earlier time even though thresholds were already just as severely altered as at 30 days. These findings suggest that tone-induced hyperactivity in the DCN is an abnormality with an onset that is delayed relative to the appearance of neural threshold shifts. Thus, if changes in spontaneous activity are related to changes in threshold, it is likely that this effect is a secondary one involving processes which are more subtle and more plastic than those leading to threshold shifts.

The suggestion that hyperactivity represents a form of physiological plasticity in the DCN is in line with previous reports that certain changes in central auditory structures vary depending on the recovery time following peripheral insult (Sasaki et al., 1980; Kim et al., 1997; Potashner et al., 1997; Suneja et al., in press). Our results suggest that one way in which the central auditory system is plastic is in its capacity to readjust its level of resting activity following the more immediate effects of cochlear insult. The level to which this activity is reset may depend on the type of insult to the cochlea, whether it is complete or partial, and whether it involves permanent damage to hair cells and/or degeneration of auditory nerve fibers. Future studies aiming to address these issues and to define the time course and mechanisms underlying these readjustments will contribute greatly to an understanding of central auditory plasticity.

Such studies will also likely yield important clues concerning mechanisms underlying tinnitus. We previously hypothesized that tone-induced hyperactivity may be an important neural correlate of tinnitus. In support of this hypothesis, we cited the observation that tone-induced hyperactivity in the DCN resembles the increase in activity which is evoked in the DCN of normal, unexposed animals by moderate-level tonal stimulation (Kaltenbach and McCaslin, 1996a). This suggests that, one month after intense tone exposure, the DCN might be signaling the presence of a sound in the absence of stimulation. It has also been reported that the severity of tinnitus in humans can be modulated by applying electrical stimuli directly to the DCN surface (Soussi and Otto, 1994). These observations taken together are consistent with the view that changes in the levels of activity in circuits within the DCN may play a role in the generation of tinnitus.

A key question which is raised by the present findings is whether tinnitus resulting from intense sound exposure shows a delay in its onset relative to hearing loss similar to that shown by tone-induced hyperactivity in the DCN. The onset time of tinnitus after intense sound exposure is not well documented in the literature. However, delays in noise-induced tinnitus onset have been reported (Axelsson and Barrenas, 1992). Future studies defining the temporal relationship of tinnitus onset to the time of noise exposure and its relationship to threshold shift would be invaluable in providing a foundation for comparison with the results of the present study.

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References

Kaltenbach, J.A., McCaslin, D., 1996b. Temporal plasticity of
Kaltenbach, J.A., Godfrey, D.A., McCaslin, D.L., Squire, A.B.,
tonotopic map of the dorsal cochlear nucleus following induction
of cochlear lesions by exposure to intense sound. Hear. Res. 59,
213–223.
Kaltenbach, J.A., Lazor, J., 1991. Tonotopic maps obtained from the
surface of the dorsal cochlear nucleus of the hamster and rat.
Hear. Res. 51, 149–160.
Kaltenbach, J.A., McCaslin, D., 1996a. Increases in spontaneous ac-
tivity in the dorsal cochlear nucleus following exposure to high
intensity sound: a possible neural correlate of tinnitus. Aud. Neuro-
sci. 3, 57–78.
Kaltenbach, J.A., McCaslin, D., 1996b. Temporal plasticity of
differences in the dorsal cochlear nucleus following exposure to in-
tense sound. ARO Abstr. 19, 168.
Kaltenbach, J.A., Godfrey, D.A., McCaslin, D.L., Squire, A.B.,
1996a. Changes in spontaneous activity and chemistry of the co-
chlear nucleus following intense sound exposure. In: Reich, G.,
Vernon, J. (Eds.), Proceedings of the 5th International Tinnitus
Kaltenbach, J.A., Meleca, R.J., Falzarano, P.R., 1996b. Alterations in
the tonotopic map of the cochlear nucleus following cochlear dam-
age. In: Salvi, R.J., Henderson, D., et al. (Eds.), Plasticity and
Regeneration of the Auditory System. Thieme, New York, NY,
317–332.
brainstem of the chinchilla after auditory overstimulation.
and spontaneous activity in the auditory system. In: Vernon, J.A.,
Moller, A.R. (Eds.), Mechanisms of Tinnitus. Allyn and Bacon,
Boston, 95–100.
Libermann, M.C., Dodds, L.W., 1984. Single-neuron labeling and
chronic cochlear pathology. II. Stereocilia damage and alterations
of spontaneous discharge rate. Hear. Res. 16, 43–53.
Libermann, M.C., Kiang, N.Y.-S., 1978. Acoustic trauma in cats:
Cochlear pathology and auditory nerve activity. Acta Otolaryn-
gol. Suppl. 358, 5–63.
Lonsbury-Martin, B.L., Martin, G.K., 1981. Effects of moderately
intense sound on auditory sensitivity in rhesus monkeys: behav-
National Academy Press, 1–150.
Meleca, R.J., Kaltenbach, J.A., Falzarano, P.R., 1997. Changes in the
tonotopic map of the dorsal cochlear nucleus in hamsters with hair
cell loss and radial bundle degeneration. Brain Res. 750, 201–213.
Potashner, S.J., Suneja, S.K., Benson, C.G., 1997. Regulation of
D-aspartate release and uptake in adult brain stem auditory nuclei
after unilateral middle ear ossicle removal and cochlear ablation.
eral partial cochlear lesions in adult cats on the representation of
lesioned and unlesioned cochleas in primary auditory cortex.
Robertson, D., Irvine, D.R.F., 1989. Plasticity of frequency organiza-
tion in auditory cortex of guinea pigs with partial unilateral deaf-
Salvi, R.J., Saunders, S.S., Gratton, M.A., Arehole, S., Powers, N.,
1990. Enhanced evoked / response amplitudes in the inferior colli-
culus of the chinchilla following acoustic trauma. Hear. Res. 50,
245–258.
to noise exposure. In: Hamernick, R.P., Henderson, D., Salvi, R.
(Eds.), New Perspectives on Noise-Induced Hearing Loss. Raven
Suneja, S.K., Potashner, S.J., Benson, C.G., 1998. Plastic changes in
glycine and GABA release and uptake in the mammalian auditory
total nuclei after unilateral middle ear ossicle removal and cochlear
ablation. Exp. Neurol., in press.
Effects of noise and ototoxic drugs on hearing. In: Ottoson, D.
(Ed.), Progress in Sensory Physiology, Vol. 9. Springer-Verlag, New
York, NY, 97–170.
2-DG uptake in the auditory system: a model for tinnitus? Neuro-
Wang, J., Powers, N.L., Hofstetter, P., Trautwein, P., Ding, D.,
Salvi, R., 1997. Effects of selective inner hair cell loss on auditory
nerve fiber threshold tuning and spontaneous and driven discharge
rate. Hear. Res. 107, 67–82.
neural coding and increase excitability in the central nervous sys-