Noise-induced hyperactivity in the inferior colliculus: its relationship with hyperactivity in the dorsal cochlear nucleus

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Submitted 12 September 2011; accepted in final form 23 April 2012

Manzoor NF, Licari F, Klapchar M, Elkin RL, Gao Y, Chen G, Kaltenbach JA. Noise-induced hyperactivity in the inferior colliculus: its relationship with hyperactivity in the dorsal cochlear nucleus. J Neurophysiol 108: 976–988, 2012. First published May 2, 2012; doi:10.1152/jn.00833.2011.—Intense noise exposure causes hyperactivity to develop in the mammalian dorsal cochlear nucleus (DCN) and inferior colliculus (IC). It has not yet been established whether the IC hyperactivity is driven by hyperactivity from extrinsic sources that include the DCN or instead is maintained independently of this input. We have investigated the extent to which IC hyperactivity is dependent on input from the contralateral DCN by comparing recordings of spontaneous activity in the IC of noise-exposed and control hamsters before and after ablation of the contralateral DCN. One group of animals was binaurally exposed to intense sound (10 kHz, 115 dB SPL, 4 h), whereas the control group was not. Both groups were studied electrophysiologically 2–3 wk later by first mapping spontaneous activity along the tonotopic axis of the IC to confirm induction of hyperactivity. Spontaneous activity was then recorded at a hyperactive IC locus over two 30-min periods, one with DCNs intact and the other after ablation of the contralateral DCN. In a subset of animals, activity was again mapped along the tonotopic axis after the time course of the activity was recorded before and after DCN ablation. Following recordings, the brains were fixed, and histological evaluations were performed to assess the extent of DCN ablation. Ablation of the DCN resulted in major reductions of IC hyperactivity. Levels of postablation activity in exposed animals were similar to the levels of activity in the IC of control animals, indicating an almost complete loss of hyperactivity in exposed animals. The results suggest that hyperactivity in the IC is dependent on support from extrinsic sources that include and may even begin with the DCN. This finding does not rule out longer term compensatory or homeostatic adjustments that might restore hyperactivity in the IC over time.

Noise exposure; tinnitus; spontaneous activity; dorsal cochlear nucleus ablation; tonotopic organization

MANIPULATIONS THAT CAUSE TINNITUS in human subjects induce dramatic changes in the spontaneous discharge patterns of neurons in auditory centers of the brain. This has been shown in animal studies using acute tinnitus inducers, such as sodium salicylate and quinine (Chen and Jastreboff 1995; Eggermont and Kenmochi 1998; Manabe et al. 1997; Ochi and Eggermont 1997), as well as inducers of chronic tinnitus, including excessive sound exposures (Brozoski et al. 2002; Kaltenbach and McCaslin 1996; Kaltenbach et al. 2000; Seki and Eggermont 2002) and platin drugs (Bauer et al. 2008; Kaltenbach et al. 2002). These treatments cause auditory centers to develop increased levels of bursting and nonbursting spontaneous activity (hyperactivity) and increased synchrony across the neural populations.

Such changes have been well characterized at the multiunit and single-unit levels in structures as diverse as the dorsal cochlear nucleus (DCN) (Brozoski et al., 2002; Finlayson and Kaltenbach 2009; Kaltenbach and McCaslin 1996; Kaltenbach et al. 2000, 2002; Middleton et al. 2011; Shore et al. 2008), the ventral cochlear nucleus (VCN) (Vogler et al. 2011), the inferior colliculus (IC) (Bauer et al. 2008; Chen and Jastreboff 1995; Dong et al. 2009, 2010a, 2010b; Jastreboff and Sasaki 1986; Ma et al. 2006; Mulders and Robertson 2009; Mulders et al. 2010, 2011; Wallhäuser-Franke et al. 2003), and the auditory cortex (Eggermont and Kenmochi 1998; Morena and Eggermont 2005, 2006; Ochi and Eggermont 1997; Seki and Eggermont 2003; Wallhäuser-Franke et al. 1996), suggesting that tinnitus may be a system-wide pathology. Indeed, functional imaging studies in both animals and human subjects with tinnitus show clear evidence of hyperactivation of centers both within and beyond the auditory system (Brozoski et al. 2007; Eichhammer et al. 2007; Langguth et al. 2006; Lanting et al. 2008; Lobaranas et al. 2008; Lockwood et al. 1998; Melcher et al. 2000, 2009; Reyes et al. 2002).

The means by which the various auditory centers acquire their aberrant activity has not yet been clarified. Abnormal activity could, in theory, be acquired by each center of the auditory system independently through the process of plasticity. Hyperactivity could emerge in these centers at the same time or at different times, but in either case, an independent mechanism would require that each level possess a capacity to readjust itself, perhaps by homeostatic mechanisms, in response to altered input from the ear. Anatomic studies do, in fact, suggest that various centers of the auditory system can undergo plastic adjustments in the balance of excitation and inhibition following alterations of cochlear input. Injury to cochlear hair cells, cochlear nerve degeneration, or functional deafferentation leads to major changes in the balance of excitatory and inhibitory synapses in brain stem auditory nuclei. These shifts apparently involve both degeneration of old and sprouting of new synapses (Bilak et al. 1997; Kim et al. 1997; Morest and Bohne 1983; Morest et al. 1997, 1998). There is also evidence for induction of hyperactivity without hearing loss, suggesting the operation of alternate plastic mechanisms that do not necessarily require a loss of input from the ear.

Shifts in the balance of excitation and inhibition in different auditory centers following noise exposure and other manipulations of peripheral input are also indicated by pharmacological and molecular studies. A weakening of inhibition in brain stem auditory nuclei following acoustic trauma or aging is suggested by decreases in glycine release, reductions of gly-
The relationship between these nuclei is particularly well suited for this study because previous studies have shown that hyperactivity in the DCN is generated by the major output neurons, the fusiform cells (Brozoski et al. 2002; Finlayson and Kaltenbach 2009; Zeng et al. 2009), which are primary sources of input to the contralateral IC. Moreover, tinnitus demonstrated using behavioral testing methods has been found to be correlated with increases in bursting and/or nonbursting spontaneous activity of DCN fusiform cells (Brozoski et al. 2002; Finlayson and Kaltenbach 2009; Kaltenbach et al. 2004) and sizeable populations of neurons in the IC (Bauer et al. 2008). We first sought to induce hyperactivity in the IC of the hamster and then directly tested the effect of removing the DCN on hyperactivity in the contralateral IC.

MATERIALS AND METHODS

Subjects. Animals were Syrian golden hamsters ranging from 70 to 120 days of age. These were housed in the animal housing facility of the Cleveland Clinic, where they were placed on a 12:12-h daily light-dark cycle. Animals were assigned to two general groups, one to be exposed to an intense sound and the other to serve as unexposed controls. Both groups were further subdivided for different experiments. The first goal was to test for successful induction of hyperactivity in the IC following intense sound exposure. The latter goal involved tests of contralateral and ipsilateral DCN ablation as well as effects of sham surgeries. All experimental procedures for these studies were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic, which follows the NIH Guide for the Care and Use of Laboratory Animals.

Sound exposure. The exposure sound was a 10-kHz continuous tone maintained at a level of 115 dB SPL for a period of 4 h. Sound exposures were conducted inside a cylindrical acrylic chamber, subdivided into four compartments by wire mesh partitions and closed at the top with a lid into which was mounted a 6-in.-diameter loudspeaker (BEYMA CP-25). The chamber was placed inside a sound insulation booth (Acoustic Systems). Before placement of the animals into the chamber, an Etymotic probe tube microphone was positioned about 1 in. above the floor of the chamber in each compartment to approximate the level where the ears of the animals would be during the exposure period. The sound was then turned on, and the voltage input to the speaker was adjusted until the tone measured 115 dB SPL at the center of each compartment. Sound levels at different positions within each of the four compartments varied by about ±6 dB. After calibration, one animal was placed in each of the four compartments, and the lid with the speaker was placed over the chamber. The sound was initially turned on at a level of 80 dB SPL for 5 min so that the animals accommodated to the presence of a moderate level tone. The sound level was then turned up in 5-dB steps every 2 min until a level of 115 dB SPL was reached. No sign that the sound caused an alteration of behavior was observed. The sound level was maintained at the 115-dB level for the remainder of the exposure period. Throughout the exposure period, the sound level was monitored to ensure constancy of the tone. After the exposure period, the animals were returned to the animal care facility and allowed a postexposure recovery period of 2–3 wk (16–23 days) before electrophysiological studies commenced.

After the recovery period, each animal was placed inside a double-walled sound attenuation room, where anesthesia was induced using a mixture of ketamine-xylazine (117 and 18 mg/kg, respectively) administered intramuscularly. A depth of anesthesia was induced at which the animal showed no toe-pincher reflexes in any limbs and no corneal touch reflex, and the breathing rate was regular and constant. The animal was then placed on a heating pad (Physitemp) that was controlled by feedback from a rectal probe sensor. A tracheotomy was performed, and the animal was mounted on a head brace. The skin
was reflected laterally, and a craniotomy was performed with the aid of a surgical microscope (Leica MZ16F) to uncover the cerebellum and the area above the midbrain. The right IC was exposed by removal of overlying portions of parietal bone and the caudal-most aspect of the right cerebral hemisphere. In experiments in which the effects of DCN ablation were studied, the DCN was uncovered as described in previous studies (Finlayson and Kaltenbach 2009). Supplements of anesthetic were administered when breathing rate exceeded 23 breaths/min or there was a return of the toe withdrawal reflex; typically, one of these signs became apparent every 45–60 min.

Electrophysiological recordings. Electrodes were micropipettes filled with 0.3 M NaCl and having tip impedances of 0.4–0.5 MΩ, sufficient to record activity from clusters of neurons (multinu- trient activity). Output from the electrode was amplified 1,000 times and band-pass filtered (300–10,000 Hz) using a WPI preamplifier (DAM80). The electrode signal was monitored on an oscilloscope and fed to a National Instruments board custom programmed using Matlab to perform measures of spontaneous activity and frequency tuning. For IC recordings, the electrode was lowered manually until its tip was just above the IC. Further movement was controlled remotely using a Narashige 3D micromanipulator placed outside the acoustic booth. A video camera mounted on top of the microscope was used to view the surface of the IC and facilitate placements of the electrode tip. In each animal, activity was mapped as a function of depth along each of three penetrations through the IC spaced 200 μm apart horizontally. For each penetration, the electrode was lowered to a depth at which responses to a search stimulus could be elicited. Search stimuli were 30-ms bursts of white noise presented at a level of 80 dB SPL. Spontaneous activity was measured every 100 μm along a depth range from roughly 1.0 to 2.6 mm below the IC surface. This range spanned a characteristic frequency range of about 2 to 32 kHz in the central nucleus of the IC (ICC).

The methods for assessing the effects of tone exposure on spontaneous activity and tuning curve thresholds were similar to those used in the DCN (Finlayson and Kaltenbach 2009). Briefly, spontaneous activity was recorded at each site by counting the number of voltage events exceeding -100 μV (~100 mV after amplification) over a period of 90 s. The measures of spontaneous activity were plotted as a function of depth, yielding an activity profile for each penetration. Activity profiles were then averaged across penetrations, yielding a mean activity profile for each animal. Comparison between mean activity profiles from exposed and control animals allowed assessment of the topographic distribution of any hyperactivity that was induced. Frequency tuning properties were determined at regular depth intervals by counting the number of voltage events in response to each of 800 tonal stimuli (16 intensities and 50 frequencies), each lasting 30 ms (5-ms rise/fall time) and separated by an interstimulus interval of 40 ms, as described previously (Finlayson and Kaltenbach 2009). These measures were used to plot response areas in which the bar height for each of the 800 stimulus conditions was proportioned to the number of counts during the stimulus period. The response areas were used to determine the characteristic frequency (CF) and CF thresholds of the recorded neurons, which provided an estimate of the degree to which neural response thresholds were shifted as a consequence of the intense tone exposure.

DCN ablation experiments. The effects of DCN ablation on activity in the right IC were studied in two different ways. The first experiment showed the time course of IC activity changes resulting from DCN ablation (see ablation methods described below). We studied the effects of three types of ablation, including thermocautery, aspiration, and freezing. Thermocautery involved touching the DCN with a filament heated to a temperature sufficient to melt the DCN surface. Aspiration involved suction of the DCN using a 25-gauge cannula connected to a vacuum pump. Freezing involved touching the DCN surface with the tip of a blunt metal probe that had been dipped into liquid nitrogen. In each case, an effort was made to leave as much of the VCN intact as possible and to avoid damaging the underlying medulla and cerebellar peduncle. In exposed animals, the IC electrode was moved to a depth where activity was found to be clearly elevated above control levels, as estimated from visual observation of electrode signal traces on the oscilloscope. Once this location was identified, the electrode was maintained at that position, and the DCN was exposed by removing the overlying portion of the cerebellum without damaging the cerebellar peduncle or any other portion of the cerebellum (e.g., paraflocculus) that was not immediately above the DCN. The number of spontaneous voltage events (~100 mV有效) was counted in successive 5-s time bins and plotted in real time for a period of 10–15 min before ablation. The DCN ablation procedure was then performed, and then activity was recorded again for 10–15 min following ablation. Such time course plots were obtained for each test of ablation in each animal. Note that the ablation procedure generally required 1–2 min and was unavoidably encumbered by sound stimulation generated by the aspirator or other surgical maneuvers. For this reason, activity recorded during the ablation period was omitted from the time course plots.

The second ablation experiment was designed to examine how DCN ablation affected the tonotopic profiles of spontaneous activity in the IC. Activity profiles were obtained by measuring activity as a function of depth at 100-μm intervals. In this experiment, most activity profiles were recorded in animals in which the DCN was either ablated or intact, but in a subset of three animals, activity was mapped as a function of depth in two penetrations before DCN ablation and then again in two different penetrations after DCN ablation; only the effect of aspiration was examined in this experiment. In a few animals, a sham procedure was performed in which the portion of the cerebellum that was normally removed to ablate the DCN was spared, but the DCN was left intact. This latter procedure allowed us to control for the effect of the invasive surgery, excluding DCN removal, on spontaneous activity. In two additional animals, the effect of ablation of the DCN on the side ipsilateral to the recorded IC was studied.

Histological analysis. After recordings were completed, each animal, while still under anesthesia, was perfused with 1.0 ml of sodium nitrate, followed by 200 ml of 0.9% saline and, finally, 200 ml of 4% paraformaldehyde fixative through the left ventricle. The brain was removed and placed in the same fixative overnight and then transferred into 30% sucrose, where it remained until the brain sank. The brain was then cut in the transverse plane into sections 40 μm thick with the use of a horizontal freezing microtome. Sections were mounted on gelatin-precoated slides and allowed to dry. The sections were then stained with thionin as described previously (Finlayson and Kaltenbach 2009). The stained sections were used to verify the effects of DCN ablation and, in selected cases, to confirm the location of the recording track.

Data analysis. Effects of tone exposure on the IC and DCN were examined by comparing the various data sets (e.g., CF vs. depth plots, mean activity profiles, CF thresholds vs. CF, etc.) in exposed animals with similar measures in controls. For the first DCN ablation experiment, plots of activity vs. time were examined individually for each animal group. The topographic distribution of any hyperactivity that was induced. Frequency tuning properties were determined at regular depth intervals by counting the number of voltage events in response to each of 800 tonal stimuli (16 intensities and 50 frequencies), each lasting 30 ms (5-ms rise/fall time) and separated by an interstimulus interval of 40 ms, as described previously (Finlayson and Kaltenbach 2009). These measures were used to plot response areas in which the bar height for each of the 800 stimulus conditions was proportioned to the number of counts during the stimulus period. The response areas were used to determine the characteristic frequency (CF) and CF thresholds of the recorded neurons, which provided an estimate of the degree to which neural response thresholds were shifted as a consequence of the intense tone exposure.
in the IC. In both tests, differences were judged to be significant if $P < 0.05$.

RESULTS

Tonotopic gradient and CF thresholds. In each animal, we mapped frequency tuning properties of neural clusters as a function of depth along the course of the penetrations through the IC, as shown in Fig. 1A. Tuning curves with clearly discernible CFs became evident at a depth of $\sim 1.0$ mm below the IC surface, indicating the upper limit of the ICC. Above this depth, responses to our noise burst search stimuli were weak or absent. As shown by the examples in Fig. 1B, taken from a single animal, tuning curves shifted from low to high frequencies with depth over a distance of 1.5 mm. The CF gradients from seven control animals, each averaged across two to three penetrations, were used to derive a mean CF gradient, which is shown in Fig. 1C. This gradient was used in subsequent experiments to localize changes in spontaneous activity induced by sound exposure. The distribution of tuning curve tip thresholds across the CF range is shown in Fig. 1D, based on tuning curves from a sample of 13 control animals. The lowermost boundary of this distribution defines the neural threshold curve. This curve has an asymmetrical U-shape and shows lowest thresholds in the range of 10–22 kHz. The majority of CF thresholds at all frequencies were within 40 dB of the lowest thresholds.

Effect of tone exposure on frequency tuning and CF thresholds. Tuning curves from the IC of a single tone-exposed animal are shown in Fig. 2A. As in control animals, the tuning curves displayed a shift toward higher CFs with depth through the IC, but the shift was less complete than that seen in the IC of control animals. This can be appreciated by inspection of Fig. 2B, which plots the CF gradients from three exposed animals. In these cases, CFs clearly increased with depth over the first millimeter of the penetration, but beyond this depth, CFs became increasingly difficult to discern due to the severity of the threshold shift and associated distortions of the tuning curve shape induced by exposure. Figure 2C shows the distribution of CF thresholds with tonotopic depth for the exposed animal group as a whole ($n = 13$). CF thresholds were within 10 dB of normal thresholds at low CFs (5–6 kHz), but above this range, thresholds shifted increasingly above control levels as the electrode was moved to deeper locations. The greatest CF threshold shifts (40–60 dB) in exposed animals were found at depths of more than 2.0 mm, which in normal animals represents CFs above 10 kHz. The 6-kHz locus was the highest frequency at which CF thresholds in exposed animals were consistently close to those in control animals and was therefore used as the common point of reference for mapping recording sites in all subsequent experiments.

Effects of tone exposure on spontaneous activity in the IC. Exposure to intense sound resulted in major elevations of spontaneous activity in the IC relative to control levels. Animals examined between 2 and 3 wk after exposure (mean 19.4 days postexposure, $n = 5$) displayed a broadly distributed pattern of hyperactivity, with levels reaching 15–23 events/s over most of the middle third of the tonotopic range (Fig. 3A). The differences between exposed and control activity levels

Fig. 1. Changes in frequency tuning with depth in the inferior colliculus (IC) of control animals. A: the path of an electrode track along a vertical penetration through the IC. B: representative tuning curves from the IC of a single control animal. Each tuning curve was based on multiunit recordings from a single site along the course of an electrode penetration through the central nucleus of the IC (ICC). C: mean ($\pm$SE) characteristic frequency (CF) vs. depth curve (tonotopic gradient) from the ICs of 7 control hamsters. D: distributions of CF thresholds across the CF range for control animals ($n = 13$). The solid curve defines the lower limit of the distribution.
were significant at all sites from the 11- to the 22-kHz loci (P < 0.05; 1-tailed t-test). This frequency range corresponds approximately to the frequency range in which CF thresholds were greatly shifted.

Figure 3B compares the mean activity levels calculated by averaging rates across all 16 tonotopic loci in the activity profiles of Fig. 3A. Activity in the exposed animals averaged 12.2 (±1.3), whereas that in controls averaged 1.8 (±1.3) events/s. This difference was highly significant (P < 0.0001; 1-tailed t-test). These results demonstrate that hyperactivity was successfully induced in the IC by intense tone exposure.

Histological results. The DCN cleaves to the brain stem surface rather loosely, and hence, its removal by aspiration was found to be relatively simple and easy to replicate across animals once the overlying cerebellar tissue was aspirated. These ablations were confirmed histologically following the recording session but are described first to establish the context in which the electrophysiological results are then described.

Examinations of the medulla in planes cut from the caudal-most level of the DCN to the rostral aspect of the anteroventral cochlear nucleus (AVCN) revealed some variation in the extent of the ablations across animals. In all cases in which ablation was attempted in exposed animals (n = 9) before a postablation recording, the removal of the DCN was found to be essentially complete (>95% loss). Examples from two animals are shown in Fig. 4. Each series of sections shows that whereas the DCN, posteroventral cochlear nucleus (PVCN), and AVCN on the right side (ipsilateral to the recorded IC) were intact, the left DCN was completely missing throughout the series while the AVCN and more than 90% of the PVCN remained intact. However, because of its close proximity, removal of the DCN also resulted in loss of the dorsal (DAS) and intermediate acoustic striae (IAS), which carry fibers to and from the DCN and PVCN, respectively. Thus the ablation procedure would have removed functional output from both the DCN and PVCN, of which only the output from the DCN projects directly to the IC (Ryugo and Willard 1985; Smith et al. 2005). The AVCN remained completely intact in 56% of the exposed animals (5/9), although one animal showed loss of about 25% of the AVCN. One additional animal showed loss of DCN, PVCN, and AVCN. In two others, it was not possible to assess whether the AVCN was intact or not, due to incompleteness of the histological series.

DCN ablation was also attempted on the right side, ipsilateral to the recorded IC of two exposed animals. In both of these animals, the right DCN and right IAS were completely removed, whereas the PVCN and AVCN were almost completely (>90%) intact.

DCN ablation was also attempted in five control animals. The DCN along with the IAS was either completely (4/5) or nearly completely (90%) lost (1/5) in these animals, but the AVCN was completely intact in all but one animal, which showed loss of 25% of the AVCN.

In the results that follow, we describe the effects of DCN ablation, focusing on the animals in which ablations eliminated all of the DCN and associated striae but left the AVCN intact. Where possible, comparisons are made with results from ani-
also caused an abrupt but transient change in respiration, which could have contributed to the decline in spontaneous activity. This problem was overcome by using the aspiration method, which resulted in an immediate reduction of activity, with the most abrupt decline occurring within seconds of the procedure, after which activity remained stable (Fig. 5B). Freezing the DCN led to a similar abrupt decline, but some degree of recovery occurred beginning about 10 min after the ablation (Fig. 5C).

Experiment 2 was performed to investigate the possibility that the changes observed in experiment 1 might have resulted from tissue movement caused by the ablation procedure, rather than from loss of the DCN. In experiment 2, the mean activity profile from the contralateral IC of the five exposed animals with intact DCNs was compared with that from five exposed animals in which the DCN was ablated minutes earlier by the aspiration method and the AVCN was completely intact. The results, presented in Fig. 6A, show a clear difference between groups. Whereas activity levels in exposed animals with intact DCNs were between 15 and 23 events/s across much of the tonotopic range (i.e., 9–20 kHz), activity in the IC of exposed animals lacking the contralateral DCN, but in which the AVCN remained intact (n = 5) and allowed similar postexposure recovery times, were consistently below 5 events/s across the same range. This decrease was statistically significant (P < 0.05; 1-tailed t-test) at seven of the tonotopic locations tested. Results similar to these were obtained in the one animal in which DCN removal was associated with loss of the AVCN (Fig. 6A). In a variation of this experiment, we compared activity profiles in the IC contralateral to the removed DCN in which the pre- and postablation recordings were performed in the same animals. The data averaged across three animals are presented in Fig. 6B and show a qualitatively similar result to that shown in Fig. 6A. Last, we tested the effect of DCN ablation on activity levels in the IC of control animals. In this experiment, the mean activity profile from control animals with intact DCNs was compared with that from five exposed animals in which the DCN was ablated minutes earlier by the aspiration method and the AVCN was completely intact. The profile from the contralateral IC of the five exposed animals in which the DCN had been ablated (n = 3). This comparison, which is shown in Fig. 6C, failed to show any significant difference between these two groups (P > 0.05), indicating that normal levels of activity survived the ablation procedure.

Effects of sham procedure. Because the DCN aspiration procedure required aspiration of the overlying portion of the cerebellum, we tested whether the loss of hyperactivity in the contralateral IC might have been related more to the loss of cerebellar tissue above the DCN than to loss of input from the DCN itself. To test this possibility, we performed a sham procedure in three tone-exposed animals in which the portion of the cerebellum overlying the DCN was removed in an identical manner as in the DCN ablation procedure, except the DCN was left intact. The results of this experiment, presented in Fig. 7A, show that removal of the cerebellar tissue above the DCN did not significantly reduce IC hyperactivity. Sham-operated animals showed similar levels of hyperactivity as exposed animals in which the cerebellum and DCN were left intact. This result indicates that loss of hyperactivity after DCN ablation was not related to loss of the overlying cerebellar tissue.

Effect of ipsilateral DCN ablation. To examine whether loss of hyperactivity in the right IC was specific to loss of input from the left DCN only or might be a product of lost input from the left DCN only. The profiles from the IC of exposed and control animals. Each curve represents the mean obtained by averaging activity profiles across animals. The most robust reductions were obtained with ablation by thermocautery (A), aspiration (B), and freezing (C). All three methods of DCN ablation resulted in a major reduction of hyperactivity in the contralateral IC. The most robust reductions were obtained with ablation by thermocautery (n = 3) and by aspiration (n = 2). Thermocautery caused an immediate decline (Fig. 5A), although this method
either side, we compared mean activity profiles from the right IC of two animals before and after ablation of the ipsilateral (right) DCN. This comparison, shown in Fig. 7B, revealed no significant effect of ipsilateral DCN ablation on IC hyperactivity. In both the pre- and postablation conditions, mean activity profiles were similar in overall shape and in the range of hyperactivity, which in both cases was between 10 and 30 events/s. A few sites showed lower postablation activity compared with their preablation levels (e.g., 0.7, 0.8, and 0.9 mm from the 6-kHz locus), but these decreases were only marginal, remained in the range of hyperactivity (10–20 events/s), and were not sufficiently large to be statistically significant ($P > 0.05$; 1-tailed $t$-test).

**Effects of DCN ablation on frequency tuning and response thresholds.** In a few animals in each group, we recorded frequency tuning curves before and after ablation with the electrode maintained at the same site for both recordings. In the examples of Fig. 8, A–C, taken from control animals in which the DCN had been ablated but the VCN remained intact, the firing rate within the boundaries of the tuning curve (i.e., the suprathreshold responses) were somewhat weaker after ablation than before, but the sharp tuning and low CF threshold

Fig. 4. Transverse sections through the medulla showing the cochlear nucleus following ablation of the left dorsal cochlear nucleus (DCN) in an exposed (left series) and control animal (right series). The positions of the various subdivisions of the cochlear nucleus [DCN, posteroventral (PVCN), and anteroventral (AVCN)] are indicated by arrows. In the 2 series shown, the DCN and associated striae [dorsal (DAS) and intermediate (IAS)] have been completely ablated, whereas the PVCN and AVCN remain intact. All 3 subdivisions are intact on the right side. These sections were obtained from the brains of animals in which recordings of spontaneous activity and tuning were previously obtained in the ICC.

Fig. 5. Time courses of activity changes during the pre- and postablation periods for each of 3 different methods of DCN ablation: thermocautery (A), aspiration (B), and freezing (C). Representative oscillographic traces taken from points marked by arrows in the time course plots in A are also shown. The duration of each trace was 200 ms. Note the reduced frequency and amplitude of multiunit voltage events in the postablation period.
recorded during the preablation period were virtually unchanged following DCN ablation. The examples from the exposed animals also showed little change after ablation compared with those recorded during the preablation period (data not shown). These results suggest that, despite the dramatic loss of hyperactivity in the IC after DCN removal, frequency tuning remained relatively unaltered by this procedure except for some slight weakening in the strength of the suprathreshold responses.

**DISCUSSION**

We have examined how spontaneous and frequency tuning properties are affected by intense tone exposure at the level of the IC. The results demonstrate induction of hyperactivity at this level and show robust effects of ablation of the contralateral but not ipsilateral DCN on IC spontaneous activity. Although DCN ablation also weakened the vigor of responses to tones somewhat, the shapes and thresholds of tunings curves were unaffected by ablation. We now discuss what these findings reveal about the relationship between hyperactivity at the IC and DCN levels and consider their functional implications.

**Induction of IC hyperactivity by intense sound exposure: comparisons with other studies.** Several previous works have examined the effects of noise or tone exposure on the levels of spontaneous activity in the IC. The first study, which was conducted in brain slices of mice, reported decreases in spontaneous discharge rates in the IC 1 wk following exposure to a 10-kHz tone for 3 h (Basta and Ernest 2004). However, an in vivo study, which was conducted in mice 13–96 days following a 1-h exposure to narrow band noise centered on 16 kHz (103 dB SPL), showed an increase of mean spontaneous rates of IC neurons tuned to frequencies near the exposure frequency (Ma et al. 2006). A series of more recent in vivo studies in

**Fig. 6. Effect of DCN ablation on spontaneous activity profiles recorded in the contralateral IC.** A: comparison of activity profiles from the IC of exposed animals with input from the DCN intact (filled squares) and those in which the contralateral (contra; left) DCN was ablated either without (open inverted triangles) or with loss of the AVCN (filled diamonds). The comparison shows a complete loss of hyperactivity in the IC following both types of lesions. B: similar comparison as in A but for an experiment in which pre- and postablation recordings were performed in the same animals. For all comparisons shown, the DCN was ablated by the aspiration method. Each point represents the mean ± SE of activity profiles from all animals in the sample, whose size (n) is indicated. C: comparison of activity profiles from control animals with and without the DCN.

**Fig. 7. Effects of various control manipulations on IC hyperactivity.** A: comparison of spontaneous activity profiles from the IC recorded from exposed animals before and after aspiration of the portion of the cerebellum overlying the left DCN but with the DCN intact. Both sets of data were obtained from the same animals. B: activity profiles examined before and after ablation by aspiration of the right DCN (ipsilateral to the IC recordings). Each point represents the mean ± SE of activity profiles from all animals in the sample, whose size (n) is indicated.
The increases in activity observed in multiunit recordings reflect an important issue raised by our results is whether recordings. Which measured between 15 and 20 events/s. Similar to those observed by Mulders and Robertson (2009), clearly elevated (15–23 event/s). Our rates at 2–3 wk were in exposed animals, activity at 2–3 wk postexposure was levels of activity were generally less than 7 events/s, whereas Mulders and colleagues. As in the guinea pig studies, control rats showed increases in the shape of spike waveforms. Overall, the results of that study showed that the hyperactivity observed in the DCN at the multiunit level after noise exposure reflects an increase in the spontaneous discharge rates of single units, and among the units that become hyperactive, fusiform cells figure prominently. Further evidence that fusiform cells become hyperactive following intense sound exposure has also been presented by others (Brozoski et al. 2002; Dehmel et al. 2012; Li et al. 2011; Pilati et al. 2011; Shore et al. 2008). A single exception that did not show increases in activity of any cell type in the DCN following noise exposure was a study conducted in decerebrate animals (Ma and Young 2006).

Loss of IC hyperactivity after DCN ablation: could it be an artifact? The abolishing effect of DCN ablation on IC hyperactivity must be interpreted with caution, because nonsynaptic influences related to the ablation procedure could influence the discharge rates of IC neurons. We considered the possibility that the sudden decline of IC activity after DCN ablation might have resulted from a shifting of the tissue relative to the electrode tip, which could have led to loss of contact between the electrode and the hyperactive neural clusters being recorded. Although this explanation could explain the results of experiment 1 (Fig. 5), in which pre- and postablation activity were recorded with the electrode at a single position, such an explanation is almost certainly ruled out by the results of experiment 2, showing that hyperactivity was abolished not just at a single tonotopic locus but across the entire tonotopic range of the IC (Fig. 6, A and B). Our DCN ablation procedures required aspiration of the overlying cerebellum, so a second possibility considered was that the loss of IC hyperactivity might have reflected loss of input from the portion of the cerebellum that was removed. This possibility is dubious since we found that a sham operation involving removal of the portion of the cerebellum overlying the DCN, while leaving the DCN intact, did not result in loss of IC hyperactivity in tone-exposed animals (Fig. 7A). A third possible artifact considered was that the ablation procedure might have caused regional injury to the brain stem, which could have spread to the IC. This explanation seems unlikely, because IC hyperactivity was not abolished by ablation of the DCN on the side ipsilateral to the recorded IC, despite its closer proximity (Fig. 7B). Moreover, injury to the IC would likely have resulted in upward shifts in tuning curve thresholds; our results, however, showed that ablation of the DCN had virtually no effect on tuning curve shapes or CF thresholds of IC neurons (Fig. 8).

A fourth possible artifact considered was that the loss of IC activity following DCN removal might have resulted from a functionally compromised state of descending neurons in the
IC caused by destruction of their axonal endings in the DCN. Various descending projections from the IC to the cochlear nucleus have been described (Alibardi, 2002, 2004; Coomes and Schofield 2004; Itoh et al. 1987; Malmierca et al. 1996; Okoyama et al. 2006; Schofield 2001; Shore et al. 1991; Weinberg and Rustioni 1987), and some of these terminate in the DCN. Because descending projections from the IC connect heavily with both the ipsilateral and contralateral DCNs, one would have expected by this mechanism that ablation of either ipsilateral or contralateral DCN would have caused loss of IC hyperactivity. The fact that IC hyperactivity was abolished by contralateral but not ipsilateral DCN ablation is inconsistent with this expectation. Moreover, it has been shown that neurons in the IC remain spontaneously active in brain slices, even though descending projections from the IC to the DCN have been severed (Basta and Ernest 2004). Taking these findings together, it seems unlikely that the loss of hyperactivity in the IC was related to the loss of descending axons originating in the IC.

Mechanisms underlying the loss of IC hyperactivity after DCN ablation. Interpretation of the mechanism by which DCN ablation eliminates hyperactivity in the IC must take into consideration the fact that the DCN influences multiple pathways, and therefore, its removal would be expected to have a myriad of effects on the IC, some direct and some indirect. The simplest and potentially most powerful effect would involve the direct input to the ICC from the DCN itself, because this structure is known to become hyperactive following noise exposure identical to that used to induce hyperactivity in the IC. Fusiform cells of the DCN project directly to the contralateral ICC (Osen 1972; Ryugo and Willard 1985; Smith et al. 2005) and are known to be an important source of hyperactivity following noise exposure (Brozoski et al. 2002; Finlayson and Kaltenbach 2009; Shore et al. 2008). Therefore, removal of input from hyperactive fusiform cells would be expected to reduce tonic hyperactivity inputting to ICC neurons, and this could account for much, if not most, of the postablation decline in IC activity.

However, the possibility must also be considered that the loss of hyperactivity in the ICC could reflect loss of hyperactive input not only from the DCN but also from the VCN. In our experiments, every effort was made to minimize damage to the VCN subdivisions, but unavoidably, the dorsal-most portion of the PVCN and IAS were removed, indicating that any inputs to the ICC from the PVCN that might have become hyperactive also would have been removed along with those from the DCN. A recent investigation has shown that some neuronal types in the VCN do indeed become hyperactive following intense noise exposure (Vogler et al. 2011), and although the VCN subdivisions in which this hyperactivity occurs were not specified, statistically significant hyperactivity was found among only two unit types, including primary-like and onset responders. Onset responses occur primarily in the PVCN and have been associated with octopus cells (Godfrey et al. 1975; Rhode and Smith 1986), which project onto neurons, via excitatory synapses, in the contralateral ventral nucleus of the lateral lemniscus (VNLL) (Cant and Benson 2003; Schofield and Cant 1997; Smith et al. 2005). However, because the VNLL is mainly inhibitory to ICC neurons (Nayagam et al. 2005; Paolini et al. 2004), removal of PVCN output through the IAS would have resulted in disinhibition of ICC neurons, which would have at least partially antagonized the decrease in activity caused by removal of DCN input. Although T-stellate cells of the AVCN and PVCN project to the ICC and VNLL, these cells are characterized by chopper responses (see Oertel et al. 2011), a pattern that was not found by Vogler et al. to become hyperactive after noise exposure; removal of their input to the ICC would therefore not seem likely to abolish hyperactivity in the ICC following our ablation procedure. The other cell type that was found by Vogler et al. to become hyperactive after noise exposure had primary-like responses to tones. These are usually associated with bushy cells in the AVCN and PVCN, which project to the superior olivary complex (SOC). The one case in which the AV CN was completely removed along with the DCN/IAS did not yield results different from those in which the AVCN was left intact, suggesting that loss of these cells does not further reduce spontaneous activity in the ICC. Even if AVCN neurons become hyperactive after noise exposure (Vogler et al. 2011), because some AVCN neurons excite the IC while others excite neurons in the VNLL, which inhibits the IC, the loss of hyperactive neurons in the AVCN would likely have a weaker effect on the IC than removing the input to the IC from the DCN. Removal of neither the AV CN nor PVCN would therefore be expected to produce the dramatic loss of hyperactivity in the IC observed in the present study.

Loss of the DCN could also have a number of other indirect effects that could alter spontaneous activity in the ICC. Some of these effects could further decrease ICC activity, whereas others would have the opposite effect. For example, tuberulous cells in the DCN inhibit bushy and stellate cells in the AVCN (Zhang and Oertel 1993). T-stellate cells project directly to the contralateral ICC as well as indirectly to the ICC via the VNLL (see review by Oertel et al. 2011). DCN ablation would thus eliminate the inhibition of stellate cells in the AV CN, which would increase direct excitatory input from T-stellate cells to the ICC, further raising activity levels. However, because stellate cells also project to the VNLL, loss of inhibitory input from DCN tuberulous cells to T-stellate cells would also increase excitatory input to neurons in the VNLL that inhibit the ICC, further decreasing ICC activity. One could similarly speculate that other pathways could be involved, such as the SOC or even the auditory cortex. Thus there is potentially more than one pathway that might contribute to the balance of excitation and inhibition in the IC. Nevertheless, the fact that hyperactive cell populations that originate in the DCN project directly to the contralateral ICC suggests that loss of direct input from these sources is likely to be a major, although not necessarily the exclusive, factor leading to the loss of hyperactivity in the IC following DCN ablation.

DCN ablation and IC frequency tuning. Aside from a slight weakening of the response magnitude, frequency tuning remained virtually unaltered from its preablation configuration after the DCN was removed. This finding is important for several reasons. First, as discussed above, it indicates that despite loss of input from the DCN, the IC maintained its functional integrity, ruling out inflammation or spread of injury to the IC from the DCN as explanations of reduced IC spontaneous activity. Second, it demonstrates that the mechanisms that determine spontaneous activity of IC neurons are separate and independent from those that determine thresholds and...
tuning properties. This is consistent with previous evidence that although the two alterations are frequently seen together, the onset of hyperactivity does not always coincide with the onset of threshold shift (Kaltenbach et al. 1998) and can even develop following recovery from threshold shift (Brozoski et al. 2002). Third, it shows that unlike IC spontaneous activity, frequency tuning in the IC is derived indirectly from the cochlea via inputs from multiple converging sources, not only from the DCN but also from other nuclei, such as the VCN and the SOC. (For a description of these inputs, see Cant and Benson 2003). Given these multiple inputs, one might expect that loss of the DCN would have only a minimal effect on IC tuning. The fact that the strength of suprathreshold responses of the IC was sometimes weakened but not enhanced following DCN ablation is consistent with this view.

Implications. Our results have several important functional implications. First, they are consistent with the conclusion, drawn from other evidence (Brozoski et al. 2002; Finlayson and Kaltenbach 2009; Shore et al. 2008), that principal cells of the DCN, especially fusiform cells, are an important source of hyperactivity in the DCN following noise exposure. This does not rule out other possible cell types within the DCN or VCN as generators of hyperactivity, but it does suggest that it is primarily the principal cells of the DCN that drive hyperactivity in the IC.

A second implication relates to the origin and mechanism of IC hyperactivity and, specifically, its relationship with the loss of inhibition, that have been found in the IC following noise exposure (Abbott et al. 1999; Dong et al. 2010a, 2010b; Milbrandt et al. 2000). The finding that IC hyperactivity was abolished by DCN ablation demonstrates that IC hyperactivity is, to a large degree, dependent on extrinsic inputs to the ICC, rather than being exclusively intrinsic in origin, and that these extrinsic inputs are highly dependent on the functional status of the DCN. This view is consistent with the finding that IC neurons in brain slices, which remove afferent input from other levels of the auditory system, do not show evidence of hyperactivity after noise exposure (Basta and Ernst 2004). If hyperactivity depends on afferent inputs from lower levels of the auditory system, does this necessarily imply that hyperactivity in the IC is independent of local reductions of inhibition? Although our results might not seem consistent with a direct causative role of reduced local inhibition in the emergence of hyperactivity in the IC, loss of local inhibition could indirectly lead to hyperactivity of IC neurons by rendering them more excitable in response to spontaneously active afferent inputs. A heightened state of excitability is demonstrated by the fact that IC neurons often develop enhanced responses to sound after acoustic trauma (Salvi et al. 1999, 2000; Willott and Lu 1982). It seems equally possible that this heightened excitability might also be manifest as an increase in activity in response to spontaneously active inputs from lower auditory centers. Yet another possibility is that loss of local inhibition does not itself indirectly cause hyperactivity but may instead be a secondary consequence of hyperactivity, reflecting degeneration of inhibitory interneurons due to excitotoxic injury induced by hyperactive inputs. Future work is obviously necessary to clarify these issues.

A third implication of our results concerns the relevance of diminished activity in the DCN and IC to tinnitus. If hyperactivity at these levels contributes to tinnitus, then the possibility is raised that DCN ablation might have the effect of attenuating or even abolishing tinnitus. However, much remains to be established before DCN ablation can be considered as a potential intervention for tinnitus. Of foremost importance, it is necessary to reconcile the present findings with a previous study that failed to show a reduction of tinnitus in animals examined weeks following DCN ablation (Brozoski and Bauer 2005). The simplest explanation is that the absence of an effect of DCN ablation on tinnitus might have reflected the incomplete nature of the DCN lesions in the study by Brozoski and Bauer. In their study, the DCN was considered accurately lesioned, even when as much as 40% of the DCN remained intact. Failure to eliminate the entire DCN could preserve enough hyperactivity to maintain tinnitus. Other possible explanations are that tinnitus-related activity changes involve auditory centers other than the DCN and IC, such as the VCN or auditory cortex, or that the behavioral test employed may not have been sensitive enough to detect low levels of tinnitus having a brain stem origin which might have been abolished. Finally, one cannot discount the possibility that homeostatic mechanisms, as described by Schaeette and Kempter (2006), might have compensated for any acute loss of hyperactivity that DCN ablation might have caused. In line with this view, earlier works have shown that deafferentation of central auditory centers leads to long-term effects that are very different from those observed shortly after the deafferenting procedure (Kim et al. 1997; Most et al. 1997; Muly et al. 2004; Sunje et al. 1998a, 1998b). Studies examining the time course of the effects of complete DCN ablation on IC or cortical activity or on tinnitus would be of great value in bringing clarity to these issues.

GRANTS

Support for this study was provided by National Institute of Deafness and Other Communications Disorders Grant R01 DC009097.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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Effects of intense tone

Jin YM, Godfrey DA, Wang J, Kaltenbach JA.


