Responses of Ventral Cochlear Nucleus Neurons to Contralateral Sound After Conductive Hearing Loss

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Submitted 19 April 2005; accepted in final form 3 August 2005

Sumner, Christian J., Debora L. Tucci, and Susan E. Shore. Responses of ventral cochlear nucleus neurons to contralateral sound after conductive hearing loss. J Neurophysiol 94: 4234–4243, 2005.—Conductive hearing loss (CHL) is an attenuation of signals stimulating the cochlea, without damage to the auditory end organ. It can cause central auditory processing deficits that outlast the CHL itself. Measures of oxidative metabolism show a decrease in activity of nuclei receiving input originating at the affected ear but, surprisingly, an increase in the activity of second-order neurons of the opposite ear. In normal hearing animals, contralateral sound produces an inhibitory response to broadband noise in approximately one third of ventral cochlear nucleus (VCN) neurons. Excitatory responses also occur but are very rare. We looked for changes in the binaural properties of neurons in the VCN of guinea pigs at intervals immediately, 1 day, 1 wk, and 2 wk after the induction of a unilateral CHL by ossicular disruption. CHL was always induced in the ear ipsilateral to the VCN from which recordings were made. The main observations were as follows: 1) ipsilateral excitatory thresholds were raised by at least 40 dB; 2) contralateral inhibitory responses showed a small but statistically significant immediate decrease followed by an increase, returning to normal by 14 days; and 3) there was a large increase in the proportion of units with excitatory responses to contralateral BBN. The increase was immediate and lasting. The latencies of the excitatory responses were at least 6 ms, consistent with activation by a path involving several synapses and inconsistent with cross talk. The latencies and rate-level functions of contralateral excitation were similar to those seen occasionally in normal hearing animals, suggesting an upregulation of an existing pathway. In conclusion, contralateral excitatory inputs to the VCN exist, which are not normally effective, and can compensate rapidly for large changes in afferent input.

INTRODUCTION

Chronic conductive hearing loss (CHL) results from outer or middle ear abnormalities that impair the conduction of sound to the inner ear without compromise of the auditory end organ. These peripheral deficits are associated with impairments of central auditory processing (Hall et al. 1995; Moore et al. 1991) observed in some subjects long after hearing thresholds return to normal (Downs 1985; Ruben et al. 1984; Vernon-Feagans 1999).

Binaural processing may be especially affected by CHL. This is reflected in poor performance on masking level difference (MLD) tasks, which in normal hearing subjects demonstrate increased signal detectability when signal or masker phase differences are introduced between the ears. Poorer performance on MLD tasks can persist for ≥2 yr after absolute thresholds return to normal (Hall et al. 1995; Pillsbury et al. 1991). Further, children with conductive hearing impairment consistently show increased latencies and abnormalities in binaurally evoked auditory brain-stem–evoked responses (Folsum et al. 1983; Gunnarson and Finitzo 1991; Hall and Grose 1993).

Changes as a result of unilateral CHL are found in many nuclei involved in binaural processing (Clerici and Coleman 1986; Clifton and Silverman 1978; Feng and Rogowski 1980; Knudsen 1999; Moore et al. 1989; Silverman and Clifton 1977; Smith et al. 1983). In the ipsilateral cochlear nucleus (CN) CHL results in a marked decrease in neuronal activity as demonstrated by decreases in cytochrome oxidase and 2-deoxyglucose activity in the ipsilateral anteroventral cochlear nucleus (AVCN). In contrast, metabolic activity increases in the opposite, contralateral AVCN (Tucci et al. 1999). Mean AVCN neuronal area after unilateral CHL in rat decreases ipsilaterally to the manipulation, but increases contralateral to the manipulation (Coleman and O’Connor 1979). Thus, the altered afferent input that results from a unilateral conductive impairment produces changes in the symmetry of binaural processing, starting at the level of the CN.

The CN is the first site in the central auditory system where convergence of binaural information occurs. Interaction between the cochlear nuclei can take place by way of the commissural pathway (Cant and Gaston 1982; Shore et al. 1992) or by descending inputs from the superior olivary complex (SOC) and inferior colliculus (IC) (Shore et al. 1991; Spangler et al. 1987). Shore and colleagues (2003) demonstrated, in VCN of the normal hearing adult guinea pig, that contralateral sound stimulation produces inhibitory responses in approximately 30% of neurons and occasional excitatory responses.

The present study used a similar paradigm to study the effect of conductive impairment on binaural interaction measured at the level of the VCN. It was hypothesized that the ratio of inhibitory to excitatory responses may be altered. The contralateral responses in the guinea pig CN were measured at various intervals after a conductive hearing loss induced by disruption of the ossicles. These results were compared to those in normal animals. The changes observed are consistent with the metabolic studies and support the hypothesis that contralateral pathways react to compensate for an ipsilateral hearing loss.
### METHODS

#### General experimental design

Multunit responses of the VCN to binaural acoustic stimulation were recorded in guinea pigs with normal hearing and after a conductive hearing loss (CHL) in the ear ipsilateral to the CN from which recordings were taken. Responses from CHL animals were obtained immediately or 1, 7, or 14 days after the CHL. Experiments were run in three separate sets. Each set included one animal for each of the times after CHL, making a total of 12 animals. Recordings from normal hearing animals were made over a more extended period, combined with recordings from other studies.

#### Animal preparation

Male and female adult pigmented guinea pigs (NIH outbred strain) weighing 250–400 g were used in this study. All animals, including CHL animals before the hearing loss, had normal Preyer’s reflexes. All procedures were performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH publication No. 80-23) and guidelines provided by the University of Michigan (University Committee on Use and Care of Animals; UCUCA).

Ossicular disruption was always performed on the left ear. Guinea pigs that received CHL surgery were anesthetized with ketamine (120 mg/kg) and xylazine (16 mg/kg). The tympanic membrane (TM) was visualized through the ear canal using a microscope. Jeweler’s forceps were used to puncture the TM, and the malleus was grasped and rotated to dislocate it from the incus. Animals recovered under supervision and were maintained in the animal facility until the appropriate survival time had elapsed. Unit responses were collected in acute anesthetized preparations. Recordings were always made in the left VCN, ipsilateral to any CHL. The guinea pigs were anesthetized with ketamine (120 mg/kg) and xylazine (16 mg/kg). Additional anesthetic was administered as necessary. Animals were held in a stereotaxic device (Kopf) with hollow ear bars. Rectal temperature was monitored and maintained at 38 ± 0.5°C with a thermostatically controlled heating pad. The bone overlying the cerebellum and posterior occipital cortex was removed and a small amount of cerebellum was aspirated to reveal the surface of dorsal cochlear nucleus (DCN) or, more rostrally, the VCN. A multichannel recording electrode was mounted to the stereotaxic device and inserted into the VCN, either directly or through the DCN.

#### Data collection

All recordings were made in a sound-attenuating double-walled booth. Sixteen-channel silicon electrodes fabricated by the University of Michigan Bioengineering Department enabled us to record simultaneously from many units. The 16-channel multielectrode array was connected by a 16-channel amplifier to a Plexon data-acquisition system. Signal input boards provided programmable gain, filtering (1,000–32,000 Hz), filter bandwidth 300–10 kHz) and analog-to-digital (A/D) conversion, with a 12-bit resolution and a sample rate of 40 kHz. Digital signal processor boards performed spike waveform capture and on-line sorting. The acquisition process was controlled from a Pentium II PC running Windows NT.

Acoustic stimuli were delivered to the ears by Beyer dynamic earphones coupled to the hollow ear bars using TDT system II hardware for D/A conversion and analog attenuation. Digital signals were generated and delivered to the TDT hardware by a Pentium PC, using the TDT software package sigPlay32. Stimuli were generated using a sampling rate of 100 kHz at 16-bit resolution. Equalization to correct for the system response was performed in the digital frequency domain. The stimulus variable sequences in pseudorandom order were generated from within MATLAB.

Binaural stimuli were used to assess the effects of contralateral sound on sound evoked and spontaneous activity of VCN neurons. Each stimulus was repeated either 50, 100, or 200 times. Responses were also recorded to ipsilateral BBN alone. A 50-ms ipsilateral noise burst was presented at sound levels from silence to 80 dB SPL in 10-dB increments. Each level was repeated 20 times. In some experiments, an ipsilateral-only condition was interleaved with the binaural stimulus and was presented at the same levels and repetitions as the contralateral noise to yield accurate ipsilateral latencies.

Data were obtained from a succession of electrode penetrations in each animal. For each penetration the recording probe tip was advanced 2–3 mm below the surface of the CN in a ventrorostal direction. Recording sites spanned 1.5 mm (100-μm spacing of recording sites) from the tip of the probe. Thus we were able to sample from much of the depth of the CN without moving the probe. The electrode was placed in a different location on the surface for each penetration, to sample behavior from different regions of the VCN.

#### Data analysis

The data analysis was performed using a custom toolbox in MATLAB both during and after the experiments. This system generated poststimulus time histograms (PSTHs), rate-level functions (RLF), and thresholds. A response threshold was taken to be the (linearly interpolated) sound level at which the difference in the mean spike rate between the driven response and the spontaneous activity satisfied a Student’s t-test for statistical significance at a level of P < 0.01. This algorithm gave reliable thresholds that agreed closely with visual inspection of PSTHs and RLFs and was able to detect both net excitation and inhibition at the same recording site. The ipsilateral BBN sequence was played both before and after the binaural stimulus. On occasion responses could change during a penetration. When the thresholds were similar (≤10 dB) they were averaged. On the odd occasion when there was a greater difference, the data were discarded.

All unit sorting on the basis of spike waveform was done on-line, and no attempt was made to isolate single-unit waveforms. Thus all
the data presented here are considered to be multunit. The trigger thresholds for spike detection were set by the acquisition software automatically to be $3.8 \times$ the root mean square (RMS) of the noise after removal of the detected spike waveforms.

**Histology**

The location of the recording electrode in the cochlear nucleus was verified post mortem. To mark electrode tracks, the recording electrodes were dipped in 10% di-I (1,1-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate, Molecular Probes), before being inserted into the brain. The brain was subsequently cryosectioned at $40–60 \mu m$ and examined under epifluorescence for evidence of recording electrode locations. Locations ranged throughout VCN.

**Controls and correcting for acoustic cross talk**

A concern when conducting binaural experiments is cross talk: signals can stimulate the opposite ear directly, without any conduction by a neural pathway. This can occur by bone conduction, air conduction, or by vibration of the supporting apparatus (Gibson 1982). To assess cross talk in our setup we performed several control experiments in normal hearing animals. The responses of VCN units to contralateral stimulation were assessed in individual animals before and after ablation of the contralateral cochlea. Any excitation synchronized to the contralateral stimulus observed under the latter condition must necessarily be ascribed to acoustic cross talk because there would be no commissural or descending neural pathway for activation of CN cells by the contralateral sound. After cochlear destruction, all inhibition disappeared and the number of units in which excitation occurred increased, suggesting that cross talk excitation is normally masked to some extent by the inhibition. The level at which excitation occurred was around 70 dB SPL.

In this study we have considered excitatory responses only in cases where cross talk can be ruled out (hereafter referred to as “genuine” or “real” responses). For each recording site we compared rate-level functions for noise in the ipsilateral (rlfi) and contralateral (rlfc) ear and attempted to find a value of cross talk attenuation (a) that would produce a close fit between the two functions. This was done by minimizing the following function across all signal levels (l), which gives the sum of the squared error for the fit

$$\text{err} = \sum_j [rlfi(l) - rlfc(l + a)]^2$$

This in effect meant sliding the contralateral rate-level functions along the sound level axis until it coincided with the ipsilateral function. The quality of this fit was then assessed visually. Those cases where a good fit was obtained were attributed to crossover and removed from any subsequent analysis. This method is much more accurate than using any global approximation for cross talk. In normal hearing animals excitatory contralateral responses were observed in 23% of all units. Of these responses, 97% were attributable to cross talk. The predicted cross talk attenuations had a mean of 55 dB and standard deviation (SD) of 6.4dB. This latter attenuation makes intuitive sense because with a CHL, the mechanism for stimulating the ipsilateral cochlea from either side will be by bone conduction, with a longer path for the contralateral sound. The distributions of the attenuations in the two populations were nonoverlapping. The data described subsequently are only those responses that could not be attributed to cross talk by this simple shift in dynamic range.

**RESULTS**

Figure 2 shows scatterplots of the BBN thresholds for both normal hearing and CHL animals. Normal hearing animals (left of the thick vertical line) are ordered according to the date and time of the recording and CHL animals (right of the thick vertical line) are ordered according to the time elapsed since the CHL surgery. Vertical dashed lines separate the different animals. Ticks on the horizontal axis separate electrode penetrations. For normal hearing animals there is a range of ipsilateral excitatory thresholds (black dots) clustered most densely below 40 dB SPL. Most units respond to ipsilateral

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**FIG. 2.** A rich representation of all the data showing thresholds to BBN presented in either ear. Data to the left of the thick vertical line are from normal hearing animals. These data were collected from individuals, not grouped in any particular way, over a protracted period of about 20 mo. Data to the right of the thick vertical line are CHL animals, arranged according to time since CHL. Points indicate ipsilateral excitatory thresholds (black), contralateral excitatory thresholds (red), and contralateral inhibitory thresholds (blue). Contralateral excitation attributable to crossover is not shown. Vertical dashed lines separate animals, and ticks on the horizontal axis delineate electrode penetrations. Within each electrode penetration responses to the different recording sites are displaced horizontally in order of electrode depth. Stacks of data points (black) at the top indicate for each penetration how many recording sites showed no response to ipsilateral sound at 80 dB SPL.
sound. Contralateral excitatory responses to BBN (red dots) are very rare. Note that excitatory contralateral responses attributable to cross talk are not included. Inhibitory thresholds to contralateral BBN (blue circles) vary widely and are typically higher than ipsilateral excitatory thresholds. In animals with CHL the ipsilateral excitatory thresholds are high, normally ≥60 dB SPL. In many cases no ipsilateral responses were measurable at the highest levels generated by our system (80 dB SPL). These units are stacked above the vertical axis. The contralateral excitatory thresholds in animals with CHL, by contrast, are much lower than ipsilateral excitatory thresholds in many cases. The contralateral inhibitory thresholds, as in the normal animals, are spread over a wide range of levels.

Comparing the data sets suggests two clear changes after a CHL: 1) ipsilateral excitatory thresholds are raised and 2) excitatory responses to contralateral sound are much more common. It is difficult to see from Fig. 2 whether there is any significant change in the inhibitory responses to contralateral sound. Example PSTHs of contralateral inhibitory and excitatory responses in two CHL animals are shown in Fig. 3. The left panels show a multiunit cluster response in which contralateral BBN at different levels (20–80 dB SPL) inhibits spontaneous activity. This inhibition is tonic, with quite rapid descent into inhibition. The right-hand panels show an excitatory response of a different unit cluster obtained under the identical stimulus condition to that for the left-hand panels. The PSTH looks very much like a primary-like ipsilateral response. Neither of these unit clusters responded to an 80-dB SPL ipsilateral noise.

Ipsilateral excitatory responses

Figure 4 summarizes the ipsilateral excitatory responses grouped according to time since CHL. Figure 4A shows the percentage of recording sites responding to ipsilateral sound. Above each bar are the numbers of units (responding/total number). The leftmost bar (labeled N) is for normal hearing animals. Asterisks indicate a statistically significant change in the percentage of responses compared with the normal hearing data set (P < 0.01; χ² test). The change in the percentage of units responding was statistically significant at all time periods after CHL. There is considerable variation between the different times after CHL, but no consistent trend. The variations arise from differences between individual CHL animals.

The inhibitory responses to contralateral sound are shown in Fig. 5 with the same format as that in Fig. 4. Data are grouped by time elapsed after CHL. Figure 5A shows the proportion of units responding and Fig. 5B shows the thresholds of those that do respond. Asterisks indicate groups that are significantly different in proportion and threshold (P < 0.01) to the normal hearing animals.

There is an initial reduction the amount of inhibition immediately after CHL, followed by a transient increase at 1 and 7 days.
days. The changes in inhibition are similar for both the proportion of responses and the thresholds. An increase in inhibition is evident as an increase in frequency and a decrease in thresholds, and vice versa for decreases in inhibition. The proportion of units responding and the thresholds are significantly different from those in the normal hearing data set except at 14 days after CHL.

Some caution must be exercised in interpreting these results because there was considerable variation between animals. Examination of the electrode location revealed by histological evidence and diagrammatic records of electrode location, and stereotaxic records of electrode depth, did not show any trends of response with location. This variability could not be accounted for by position within the VCN or any other experimental variable we could determine.

**Contralateral excitatory responses**

Figure 6 shows the excitatory responses to contralateral sound grouped by time elapsed after CHL. Figure 6A shows the proportion of sites responding, and Fig. 6B shows the thresholds of those that do respond. Asterisks indicate groups that are significantly different in proportion and threshold (P < 0.01) from the normal hearing animals. These data are uncontaminated by cross talk, as described in METHODS. Subsequent analysis will also show that the latencies are also not consistent with cross talk. The plot shows a very significant increase in the proportion of contralateral excitatory responses at all times after CHL. The thresholds of the excitatory responses are significantly different from the normal hearing data at 1 day after the CHL. Overall the more robust change is seen in the percentage of responses, not their thresholds.

As with contralateral inhibition, there was considerable variability between animals. This could not be accounted for by electrode location.

**Spontaneous activity**

Figure 7, A and B shows histograms of spontaneous rate (SR) found in normal animals and after CHL (all data sets), respectively. In normal animals we see a large proportion of fairly low spontaneous rates (36% have SR < 10 s⁻¹). After CHL the peak of the function shifts to 10–20 s⁻¹, and the distribution spreads to still larger SRs. The mean value reflects this shift, almost doubling from 28.3 s⁻¹ in the normal hearing animals to 50 s⁻¹ after CHL.

Figure 7, C and D shows how the SRs vary with time since CHL. Figure 7C shows the percentage of units having SR > 20 s⁻¹ vs. time elapsed after CHL. Fractions above each bar indicate the number of SR > 20 s⁻¹ (numerator) over the total number (denominator). Asterisks show those CHL groups that are significantly different from the normal animals according to a χ² test (P < 0.01). D: mean SRs and SDs vs. time elapsed since CHL. Asterisks indicate those CHL groups significantly different from the normal animals according to Dunnett’s test (P < 0.01).

**Rate-level functions**

Figure 8 shows mean rate-level functions (RLFs) for the responses to contralateral sound when there is no ipsilateral stimulus. Figure 8A shows the mean inhibitory RLFs (IRLFs) for the normal hearing animals (solid line), and the entire set of CHL animals (dashed line with crosses). Only responses that

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**FIG. 6.** Contralateral excitatory thresholds vs. time elapsed since CHL. A: percentage of responses to contralateral sound that increase in firing rate. Fractions above each bar indicate the number of recording sites showing a response (numerator) over the total number (denominator). Asterisks show those CHL groups that are significantly different from the normal animals according to Dunnett’s test (P < 0.01).

**FIG. 7.** Spontaneous activity. A: histogram of spontaneous rates (SRs) in normal hearing animals. B: histogram of SR in all animals with CHL. C: percentage of SR > 20 s⁻¹ vs. time elapsed after CHL. Fractions above each bar indicate the number of SR > 20 s⁻¹ (numerator) over the total number (denominator). Asterisks show those CHL groups that are significantly different from the normal animals according to a χ² test (P < 0.01). D: mean SRs and SDs vs. time elapsed since CHL. Asterisks indicate those CHL groups significantly different from the normal animals according to Dunnett’s test (P < 0.01).
Spike rate has been expressed as a proportion of the spontaneous rate. Both functions are monotonic and quantitatively similar. Figure 8 shows the mean functions for each period after CHL. All the functions are qualitatively similar. Immediately after CHL, there appears to be a decrease in the strength of the inhibitory responses. This difference accompanies the rise in thresholds shown in Fig. 4B.

Figure 8, C and D shows the corresponding mean contralateral excitation RLFs (ERLFs). These functions are normalized to the maximal (=1) and spontaneous (=0) rates of each unit so capture only the shape of the driven response function. Only those responses that cannot be attributed to cross talk are included. Figure 8C compares normal and CHL responses. Excitatory responses in both sets show similar monotonically increasing functions. Although these are mean data, it is common to find this shape of function at a single recording site. Figure 8D shows the ERLFs as they vary with time after CHL. There is no apparent change with time.

### Cooccurrences between different response types

A large number of the contralateral excitatory responses occur when there is no ipsilateral response to sound. This is evident in Fig. 2. Table 1 quantifies the cooccurrence of different types of response in CHL units: measurable ipsilateral excitation, contralateral excitation, and contralateral inhibition. The numbers in the row and column titles show the number of units having a given single response type. The entries in the table show the percentage of contralateral responses that occur for a given ipsilateral response type. The numbers in parentheses indicate the actual numbers of units for each combination of ipsilateral excitatory and contralateral responses. The following is evident that 1) contralateral inhibition is just as likely to occur regardless of ipsilateral excitatory responses (31%: 32%), and 2) contralateral excitation is ten times as likely to occur when there is no ipsilateral response (20%) as when there is (2%). This suggests that more profound CHL cases, in which ipsilateral responses could no longer be measured at the level tested, are more likely to be accompanied by contralateral excitatory responses. 3) Co-localization of contralateral excitation and inhibition is very rare (<1%). This may indicate that separate clusters of neurons receive only one type of input or that they mask each other. If the latter is true, both inhibition and excitation may be underestimated in the current analysis.

Table 2 is of a similar format to that of Table 1, but shows the mean SRs for units with different combinations of response. Asterisks show those groups that are significantly different from the normal SR (28.3 s⁻¹, as shown in Fig. 7), according to a $t$-test with $P < 0.01$. Several factors are apparent: 1) SRs are higher than those in normal animals; 2) SRs are higher when there is ipsilateral response than when there is not; 3) SRs are highest when there is also contralateral inhibition; and 4) SRs are lowest when there is contralateral excitation and no ipsilateral response. Overall this suggests that with a milder CHL spontaneous activity increases, and for stronger CHL this effect lessens. It also shows a dissociation of the largest changes in SR from the contralateral excitation.

### Table 1. Cooccurrence of different response types to ipsilateral and contralateral sound in VCN units after CHL

<table>
<thead>
<tr>
<th></th>
<th>No Ipsi Response</th>
<th>Ipsi Excitation</th>
</tr>
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<tbody>
<tr>
<td>No contra response</td>
<td>49% (338)</td>
<td>67% (129)</td>
</tr>
<tr>
<td>Contra inhibition</td>
<td>31% (213)</td>
<td>32% (61)</td>
</tr>
<tr>
<td>Contra excitation</td>
<td>20% (135)</td>
<td>1% (3)</td>
</tr>
<tr>
<td>Contra excitation and inhibition</td>
<td>0.3% (2)</td>
<td>0% (0)</td>
</tr>
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Values in row and column headings are the total numbers of a particular response characteristic (this includes those showing more than one type of response). Values in the table show the percentages of particular contralateral response type, given the type of ipsilateral response. The actual numbers of recording sites for each combination are in parentheses: e.g., 31% of 687 = 213 recording sites that do not respond to ipsilateral BBN show inhibitory responses to contralateral BBN.
Latencies

Figure 9 compares the latencies of the excitatory responses to BBN stimulation in either ear, in CHL and normal animals. The values are for the first peak of the excitatory response, which was located manually from each PSTH. Error bars show 1/SE. These values were derived from subsets (−1/3) of the larger data sets for which sufficient sound levels and stimulus repetitions existed for sound presented to either ear. However, because spike rates were too low to yield any measurable latencies for contralateral excitation in normal animals, more units that had responses that could not be attributed to cross talk were selected by hand from the complete normal data set.

In normal hearing animals, mean ipsilateral excitatory latencies (∘, n = 166) are in the range 2–4 ms depending on level. Latencies of cross talk responses from contralateral stimulation in normals ( qx , n = 19) are around 3.5–4 ms. This makes sense because latencies to cross talk at high contralateral levels should correspond roughly to low-level ipsilateral latencies (bone conduction time is −0.2–0.5 ms; Sohmer and Freeman 2001). Genuine contralateral excitatory latencies are much longer for normal (∘, n = 2) and thus error bars are not shown for this set and CHL (+, n = 62) animals. Although latencies were very long in normals, such latencies were also seen occasionally for CHL animals so we cannot know whether this is a reliable difference. The long latencies emphasize further that they cannot be ascribed to cross talk. For CHL animals, ipsilateral stimulation ( qx ; n = 63) produces latencies very similar to the contralateral cross talk in normals. This also makes sense because the mechanism for both is similar. The long latency of “real” contralateral excitation is inconsistent with the notion of acoustic cross talk. These responses must arrive by a neural path several synapses longer than that of ipsilateral sources.

DISCUSSION

In this study, induction of a CHL in guinea pigs produced four changes in the responses of VCN neurons to sound. First, as shown previously, thresholds to ipsilateral sound stimulation were raised by ≥40 dB. This is a predictable consequence of removing the mechanical link between the tympanic membrane and the oval window. The second major change, which has not previously been reported, was a dramatic increase in the number of VCN neurons that were excited by contralateral sound (~20%). In the normal guinea pig, VCN cells are rarely excited by contralateral sound (Shore et al. 2003). In contrast, the percentage of neurons inhibited by contralateral sound showed a statistically significant decrease immediately after the CHL, followed by a small but significant transient increase. Percentages were returning to normal values (~30%) by 2 wk post CHL. Finally, there was an increase in spontaneous firing rate. This occurred immediately and lasted for the longest post-CHL period.

Contralateral excitation

The contralateral excitation reported in the present study was a consequence of synaptic activation. This excitation could not be attributed to acoustic cross talk or bone conduction because we included only those responses that could not be accounted for by any cross talk attenuation. The remaining units had contralateral thresholds below ipsilateral thresholds. Furthermore, latencies of responses were longer than those for ipsilateral activation of the same neurons. In many cases there was no response to ipsilaterally presented sound at all. The immediacy of the changes strongly suggests an increase in the effectiveness of existing synapses. This hypothesis is supported by the occasional observation of contralateral excitatory responses in normal hearing animals (Shore et al. 2003).

The long latencies (>6 ms) of excitatory contralateral responses suggest that they are mediated by descending pathways. One possibility is the excitatory cholinergic pathway to the CN by the medial olivocochlear bundle (OCB) originating in the superior olivary complex (SOC; Benson and Brown 1996; Fujino and Oertel 2001; Godfrey et al. 1987a,b; Mulders et al. 2003; Spangler et al. 1987), primarily from the ventral nucleus of the trapezoid body (VNTB; Sherriff and Henderson 1994). However, we cannot rule out the commissural CN pathway, which probably mediates the inhibitory contralateral responses (Needham and Paolini 2003; Shore et al. 2003). A few of the neurons constituting the CN commissural pathway have anatomical characteristics indicative of excitatory neurotransmission (Alibardi 1998, 2000; Shore et al. 1992) and terminate in granule cell regions (Alibardi 2004), increasing the likelihood of slow temporal integration (Shore 1998). Furthermore, even in principal cells in the CN, temporal integration in dendrites can last 10 ms (Palmer and Winter 1996).

The fact that excitation by contralateral sound changed more dramatically than inhibition suggests that excitatory pathways to the VCN may be largely responsible for the observed changes. Recent evidence suggests that the cholinergic fibers of the OCB that innervate the CN en route to the cochlea might be upregulated after cochlear damage (Jin et al. 2005; Kraus and Illing 2005), providing further support for the VNTB as a source of the contralateral excitation. Alternatively, an increase...
in glutamatergic activity in both cochlear nuclei and also the SOC and IC after CHL (Potashner et al. 1997) is consistent with the notion that descending glutamatergic pathways mediate the contralateral excitation described in the present study. In line with this hypothesis, changes at excitatory amino acid synapses as a consequence of cochlear damage have been reported in many parts of the brain stem. In the cochlear nucleus, noise exposure causes an increase in glutamate release and a decrease in uptake (Muly et al. 2004). Also, glutamate receptors are upregulated after cochlear ablation (Suneja et al. 2000), rendering any remaining glutamatergic inputs more efficient. A similar increase has been observed in the sensitivity of cholinergic receptors after deafness in slice preparations of DCN (Chang et al. 2002). Possible sources of descending glutamatergic inputs to the CN include the contralateral CN and the inferior colliculus (Alibardi 2000; Saint Marie 1996; Shore et al. 1991).

An alternative mechanism for increasing excitation is release from inhibition, or “unmasking” of synapses (Calford 2002). Other reports of fast plasticity in the auditory system have been attributed to unmasking (Mossop et al. 2000; Snyder and Sinex 2002). Such a release from inhibition would probably have to be from tonic- rather than stimulus-driven inhibition. If such inhibition were stimulus driven, then we would expect to observe excitation in normal animals whenever there is simply no ipsilateral sound. A change to tonic inhibition might be predicted as a result of the reduced afferent spontaneous input (Cook et al. 2002). This is not easy to attribute to local inhibitory neurons in the CN that have low SRs and higher thresholds than those of the excitatory neurons (Winter and Palmer 1995). Descending sources of γ-aminobutyric acid (GABA) from the superior olivary complex (Ostapoff et al. 1990) and inferior colliculus (Alibardi 2002) are more likely candidates. In line with this hypothesis, tonic GABAergic inhibition of spontaneous activity has been observed in VCN (Ebert and Ostwald 1995).

A further possibility is that the action of olivocochlear efferents to the cochlea might also be altered after CHL, producing an increased excitation of VIIIth nerve fibers in the ear contralateral to the damaged ear (Benson and Brown 1990, 1996; Brown and Benson 1992; Brown et al. 1988, 1991; Godfrey et al. 1987a; Robertson and Winter 1988; Robertson et al. 2002). Under normal circumstances, activity of PVCN neurons activates SOC neurons on both sides of the brain, including OCB neurons that project to the cochlea on the other side and suppress AN activity (Cant and Casseday 1986; de Venecia et al. 2005; Schofield 1995; Smith et al. 1993). After CHL spontaneous AN activity decreases. Thus the driving force to OCB neurons might decrease, altering the OCB modulation of the opposite cochlea. Thus the normally suppressive action (Liberman and Brown 1986) of the OCB will be diminished, resulting in a subsequent increase in VIIIth nerve activity and possible increase in excitation of CN neurons.

**Contralateral inhibition**

In normal animals, approximately 30% of VCN neurons are inhibited by contralateral sound (Shore et al. 2003; this study). It is assumed that most of that inhibition, which is of short latency, is carried by fibers of the CN commissural glycinergic pathway (Babalian et al. 2002; Needham and Paolini 2003; Schofield and Cant 1996; Shore et al. 1992). Immediately after CHL, this percentage dropped to 10%, thereafter rising transiently at one day to higher than normal values that remained elevated at 14 days.

It is possible that these numbers underestimate the real proportion of inhibitory responses. First, some inhibition may have been masked by the increase in excitation. Second, in normal hearing animals, contralateral inhibition is easier to detect if the baseline firing rate is raised slightly by ipsilateral stimulation (Sumner and Shore, unpublished observation). In the CHL animals the loudest ipsilateral level was frequently subthreshold so there was no baseline shift with ipsilateral stimulation. This may have made the inhibition harder to detect as we could only look for changes in spontaneous activity. However, this may not have had much impact because spontaneous activity in many of these cells also increased (see Fig. 7).

After cochlear ablation, the release of glycine remains normal in the VCN, but is deficient in the DCN bilaterally after 2 days (Potashner et al. 2000). The temporary increase in contralateral inhibition we see may be partially attributable to a temporary decrease in inhibition from contralateral CN neurons by the tuberculoventral pathway (Ostapoff et al., 1999; Wickesberg et al. 1991). Similar predictions might be made for a decrease in descending inhibition from other structures. Additionally, when measured using immunocytochemistry the local increases in neurotransmitter from contralateral sources might well be hidden by dominant contrary changes occurring from the loss of afferent input.

**Spontaneous rate**

Immediately after CHL there was a significant increase in the SR of VCN neurons that declined with time but did not yet reach normal SR by 14 days. Kaltenbach and colleagues (Kaltenbach and Afman 2000; Kaltenbach and McCaslin 1996; Kaltenbach et al. 1998) reported elevated SR in the DCN after intense pure-tone exposure. It is possible that similar mechanisms are at work throughout the CN. However, there are important differences compared to our results. In DCN the SR increases over several days (Kaltenbach et al. 2000) and the cell types and intrinsic circuitry (Young 1998) in the DCN are very different from those in the VCN.

The increase in overall SR we observe in VCN is in contrast to the decrease in spontaneous afferent input (2–3 dB; Cook et al. 2002) after CHL. Like the increase in excitation, the increase in VCN SR could be a result of either increased excitation or decreased inhibition by descending pathways from the SOC. However there is a disassociation of the populations showing SR increases and contralateral excitation, so any common mechanism would have to ultimately affect different neurons in different ways.

**Implications for binaural processing during CHL in humans**

It has long been assumed that binaural processing begins in the superior olivary complex. However, recent work demonstrates that functional connections between the cochlear nuclei exist and has established that stimulation of the contralateral ear can inhibit neurons in the VCN and DCN (Needham and Paolini 2003, Shore et al. 2003). The current study extends
these findings by showing that not only does the CN play a role in binaural processing in normal hearing individuals, but that it may also be the first neural location in which binaural processing is altered after peripheral insult.

Although the binaural interaction measured with the MLD is presumed to originate in the SOC, the neural basis of this response is not known (Jiang et al. 1997), and it is possible that the CN contributes to this response. The plasticity of the binaural response reported here appears to partially compensate for the discrepancy in input between the ears after unilateral CHL. In the gerbil, 2-deoxyglucose uptake is substantially diminished in the major afferent projection from an ear with a unilateral CHL, particularly during early development (postnatal day 21). In animals with a mature auditory system (postnatal day 42), the left–right discrepancy between sides of the central auditory system is less marked (Tucci et al. 1999), perhaps reflecting the compensatory mechanisms reported in this study.

ACKNOWLEDGMENTS

We thank S. Koehler and M. Bissinger for expert help with data analysis and histology.

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GRANTS

This work was supported by National Institute on Deafness and Other Communication Disorders Grants DC-05415, DC-004825, and PO1 DC-00078.

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J Neurophysiol • VOL 94 • DECEMBER 2005 • www.jn.org


