

EXHIBIT

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Perception-based protection from low-frequency sounds may not be enough

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Hearing and perception in the mammalian ear are mediated by the inner hair cells (IHC). IHCs are fluid-coupled to mechanical vibrations and have been characterized as velocity-sensitive, making them quite insensitive to low-frequency sounds. But the ear also contains more numerous outer hair cells (OHC), which are not fluid coupled and are characterized as displacement sensitive. The OHCs are more sensitive than IHCs to low frequencies and respond to very low-frequency sounds at levels below those that are perceived. OHC are connected to the brain by type II afferent fibers to networks that may further attenuate perception of low frequencies. These same pathways are also involved in alerting and phantom sounds (tinnitus). Because of these anatomic configurations, low-frequency sounds that are not perceived may cause influence in ways that have not yet been adequately studied. We present data showing that the ear's response to low-frequency sounds is influenced by the presence of higher-frequency sounds such as those in the speech frequency range, with substantially larger responses generated when higher-frequency components are absent. We conclude that the physiological effects of low-frequency sounds are more complex than is widely appreciated. Based on this knowledge, we have to be concerned that sounds that are not perceived are clearly transduced by the ear and may still affect people in ways that have yet to be fully understood.

1 INTRODUCTION

The manner in which the inner ear responds to very low-frequency sounds is still not well characterized. The pertinent anatomy and physiology is diagrammed in Figure 1. When sounds enter the cochlea they stimulate different regions, depending on the frequency, or tone, of the sound. The basilar membrane has a low-pass filter characteristic, such that the basal turn can respond to all frequencies while higher frequency components are progressively filtered out towards the apex. The apical regions are where mechanical-to-electrical transduction occurs for

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only the low-frequency components of acoustic sound. Superimposed on the passive mechanical filtering are cochlear hair cells that amplify low-level sounds for detection. This mechanical amplification is performed by the outer hair cells (OHC) so that the signal can be detected by the inner hair cells (IHC) that are the true sensory cells of the ear. The IHCs are densely innervated by type I primary afferent fibers, which is why it is generally accepted that hearing and perception are mediated by IHC activity. An important feature of the IHCs is that their sensory stereocilia (“hairs”) do not contact the overlying tectorial membrane. They are therefore fluid coupled to the mechanical vibrations and have been characterized as velocity-sensitive, or ac-coupled¹. That is to say, high-frequency vibrations stimulate IHCs but low-frequency vibration is attenuated, making IHCs insensitive to very low-frequency sounds. This contributes to the insensitivity of mammalian hearing to very low-frequency sounds and infrasound, requiring high levels to be heard. However, this is not to say that the ear itself is insensitive to very low-frequency sounds and infrasound. The stereocilia of the OHC are directly coupled to the tectorial membrane so they receive mechanical input in a displacement-sensitive manner. In early studies, Békésy² showed that displacements of the basilar membrane by trapezoidal stimuli generated trapezoidal response potentials that were sustained for the duration of the stimulus. The OHCs are thus dc-coupled to input and therefore highly sensitive to low-frequency stimulation. The OHC are, in part, connected to the brain by the type II afferents which make up approximately 5% of the afferent fibers in the auditory nerve. Each type II fiber contacts multiple OHC. Although no one has ever reported recordings from type II fibers with infrasound stimulation, Schermuly and Klinke³ have shown that similar fibers in the bird, that innervate multiple hair cells, are highly sensitive to infrasound input.

Studies have suggested that the perception of low-frequency sounds by humans is influenced by the presence of higher-frequency sounds. Krahé^{4,5} found that the perception of low-frequency noise alone was rated to be more annoying than low-frequency noise presented with higher-frequency sounds. These studies suggest that the perceptual consequences of low-frequency sounds should not be studied without considering the combined effects of higher-frequency sounds such as those in the range of speech.

Here we report objective measures from the low-frequency regions of the cochlea. They offer support for hypotheses that the influence of high-frequency sounds on the perception of low-frequency sounds is rooted in a cochlear mechanism in which the OHCs near the apex are stimulated by low frequency sounds more intensely than previously understood.

2 METHODS

Stimulus generation and response acquisition were performed using Tucker-Davis System 3 hardware controlled by custom-written software on a personal computer. Sound stimuli were delivered in a closed system using a hollow ear bar between the transducers and the external ear canal of anesthetized guinea pigs. Full details of stimulus delivery and presentation are given elsewhere⁶. Cochlear responses were measured from 500 mM KCl-filled glass pipettes inserted into endolymph of the cochlear third turn and connected through a high-input impedance electrometer. All procedures were approved by the Animal Studies Committee of Washington University under protocols 20070147 and 20100135.

3 RESULTS

3.1 Suppression of Infrasonic-Tone-Response by a Higher-Frequency Tone

The response of the apical, low-frequency regions of the cochlea to low-frequency sounds is complex. Responses are large when the sound is dominated by low frequencies and become smaller when higher-frequency sounds are present. In Figure 2, the response to an infrasonic (5 Hz, 90 dB SPL) tone was recorded from endolymph of the third cochlear turn while a higher frequency (500 Hz) tone was superimposed after 1 second. As the level of the 500 Hz tone was increased from 50 to 80 dB SPL, the response to the 5 Hz stimulus was dramatically suppressed. Suppression of the infrasound response occurred at stimulus levels well below those that saturate the mechanical-to-electrical hair cell transducers of the inner ear (Figure 2, lower left panel), meaning that the suppression is not a result of transducer saturation.

3.2 Low-Pass Noise: A Variant of Infrasonic-Tone-Response Suppression

Responses to low-pass filtered noise were measured with electrodes located in the basal turn (sensitive to high frequency sounds) and in the third cochlear turn (sensitive to low-frequency sounds). All recordings were made with electrodes located in the endolymphatic compartment of the guinea pig inner ear. The sound stimuli used are shown in Figure 3. White noise stimuli were generated and digitally low-pass filtered with a cutoff slope of approximately 55 dB/octave. The noise was digitally generated "frozen noise" so that it had the characteristics of white noise but was exactly repeatable for each of the low-pass filtered conditions, allowing multiple responses to be time-domain averaged. The spectra here were obtained from 20 responses averaged with the noise at a level of 90 dB SPL for the 4 kHz filtered condition. The low-pass cutoff frequency of the filter was varied in half-octave steps from 125 Hz to 4 kHz. Filtered electrical signals sent to the headphone for sound stimulation are shown in the upper panel of Figure 3. These stimuli were delivered by a Sennheiser HD 580 driver and the spectra measured in the canal are shown in the middle panel of Figure 3. For each cutoff frequency the noise levels were measured either with or without filtering the microphone response with a 22 Hz high-pass filter that reduced ambient room noise. The signals were also measured with A-weighting as shown in the lower panel of Figure 3. The noise level for the 125 Hz low-pass filtered condition had an A-weighted level of 56 dB A.

Spectra shown on an expanded frequency scale (0 to 300 Hz) for three simultaneously recorded conditions are shown in Figure 4. Responses measured from the animal are expressed as dB re. 1 V where -72 dB represents $\sim 250 \mu\text{V}$ response amplitude. The microphone ear canal measurements of Figure 4, which are the same data as in Figure 3, show that the low-frequency components are indeed frozen, as all measures overlaid each other in the 20 – 100 Hz range. The right column of Figure 4 shows the average spectral level over the 12-125 Hz range for each low-pass filter condition. When measured in the basal region of the cochlea, noise with lower frequency cutoff produced larger responses in the low-frequency range. The spectral average over the 12-125 Hz range was approximately 3 dB greater for the 125 Hz cutoff noise than it was with the cutoff set to 2000 Hz, a characteristic that was not present in the simultaneously measured sound levels in the ear canal demonstrating that our inner-ear measures are not an analysis artifact. This same tendency was more pronounced when responses were measured from the third cochlear turn in that the noise with the lowest cutoff frequency generated a substantially larger response. When the response amplitude from the third turn was averaged across the 12-125 Hz range, an approximately 6 dB decline was seen between the 125 Hz and 2000 Hz low-pass filter settings. The results of similar measurements made in 5 animals are summarized in Figure 5. The responses in the low-frequency spectral region (12-125 Hz) were 8.8 dB greater

from the 125 Hz low-pass cutoff noise as compared to that from the 2 kHz or higher cutoff frequencies. In other words, *responses from low-frequency stimulus components were 2-3x greater in amplitude when high-frequency sounds were not present.*

3.3 The Effect of A-Weighting

Although the low-pass filtered noise with a cutoff frequency of 2 kHz or greater was set to 90 dB SPL, the measured sound levels decreased, as expected, for stimuli with lower cutoff frequencies as higher frequency components were filtered out. The decline with cutoff frequency, measured in dB SPL, is shown in the lower panel of Figure 3. The changes as cutoff frequency was varied become even more pronounced when the sound was A-weighted. The A-weighted level of the noise with the cutoff filter set at 125 Hz was 56 dB A.

The low frequency responses of the ear, measured as the average spectral components from 12 – 125 Hz, as a function of noise levels is summarized in the left panel of Figure 6. As level was increased, the response from the 125 Hz low-pass filtered noise was always larger than the 4 kHz low-pass filtered noise. In this plot, the sound level represents how the data were collected, based on the noise level for the 4 kHz low-pass (i.e., wide-band) condition. In the right panel, we provide a comparison of the two noise-band responses corrected by A-weighted levels. There are two major observations that result from the comparison in Figure 6 (right panel):

- 1) **Low-pass filtered noise with a cutoff frequency of 125 Hz presented at a level of ~43 dB A stimulated the apical regions of the cochlea to the same degree as noise with a low-pass cutoff frequency of 4 kHz (i.e. wide band) at a level of 90 dB A.**
- 2) **At stimulus levels above 45 dB A, 125 Hz low-pass filtered noise generated larger responses at the apical regions of the cochlea than were generated by ANY level of 4 kHz low-frequency cutoff (i.e. wide band) noise.**

4 DISCUSSION AND CONCLUSIONS

The sensitivity of the apical regions of the cochlea to low-frequency sounds, and the suppressive influence of higher frequency sounds on this response, is confirmed by this study. We have demonstrated that A-weighted noise levels of as low as 45 dB A can stimulate apical regions to the same degree as wide band noise of much higher levels, as high as 90 dB A. This study shows that it cannot be assumed that noise levels as low as 40 dB A are benign and do not cause strong stimulation of the ear. Low-frequency noise around 40 dB A undoubtedly affects the ear. If the noise consists of predominantly low frequencies, then it will induce greater stimulation of the ear than has hitherto been appreciated. The observation that responses to primarily low-frequency noise stimulation are larger and do not saturate to the degree seen when higher-frequency components are present (Figure 6) is in complete agreement to the behavior previously seen with tonal stimuli⁷. The input/output functions of cochlear responses saturated at progressively higher levels for 500 Hz, 50 Hz and 5 Hz tonal stimuli presented in quiet. This means that the largest electrical responses in the apical regions of the cochlea will occur specifically when very low-frequency sound dominates the stimulus and mid-frequency components (200 – 2000 Hz) are absent.

The responses from inside the inner ear reported here may provide a physiologic basis for Krahé's psychoacoustical studies that showed how low-frequency noises with sharp cutoff slopes

are judged to be more annoying than when presented with higher-frequency sounds, as is the case when lower-cutoff slopes are present^{4,5}. Although our studies offer support for Krahé's findings, we do not necessarily agree with his interpretation that the annoyance is mediated by primary type I afferent auditory nerve fibers. We have shown empirically that infrasound rarely leads to direct excitation of single-auditory-nerve afferent fibers⁷ and there are many other mechanisms that should not be ruled out, including:

1) Stimulation mediated by type II afferent fibers. Type II fibers innervate multiple OHC and connect to multiple cell types of the cochlear nucleus⁸ in the brainstem. These pathways may be inhibitory to conscious hearing⁹, and may also be linked to alerting and attention pathways.

2) Cochlear Fluids Disturbances. Low-frequency stimulation at non-damaging levels has been shown to result in a localized endolymphatic hydrops – a swelling of the endolymphatic inner-ear compartment – with associated basally-directed endolymph flow¹⁰. Wit et al. showed that during endolymph volume increases, pressure changes were consistent with a flow through a narrow duct (the ductus reuniens) into a more compliant chamber (the sacculus)¹¹. Histologic studies showed that the sacculus is highly compliant and is one of the first structures affected by endolymphatic hydrops¹². Low-frequency sound exposure could therefore produce saccular disturbance, with symptoms including unsteadiness and disequilibrium. Furthermore, endolymphatic hydrops has been shown to contribute to an occlusion of the cochlear helicotrema which then makes the ear approximately 20 dB more sensitive to very low-frequency sounds¹³. This leads to the possibility of a positive feedback process, with low-frequency stimulation generating hydrops that in turn makes the ear more sensitive to the low-frequency stimulation. In addition to the mechanical disturbance of the saccule caused by endolymphatic hydrops, saccular enlargement also brings the saccular membranes closer and possibly in contact with the stapes footplate which would result in more efficient, direct stimulation of the saccule.

3) Amplitude modulation of sounds in the acoustic range. We have shown that low-frequency sounds that do not directly stimulate the IHCs and primary afferents, can influence the responses of the auditory nerve (i.e., sounds that will be heard) by inducing a biological form of amplitude modulation⁷. This type of modulation occurs within the cochlea and cannot be measured with a sound level meter. Rather, because the low-frequency displacements of the basilar membrane affect the amplification properties of the OHCs, responses to high-frequency sounds are perceived as being modulated in amplitude.

It is well documented that people find noise with prominent low frequency content annoying^{4,5,14}. In the context of wind turbine noise it is known that the larger wind turbines can generate high levels of low frequency noise and infrasound^{15,16,17,18,19}. The concern arising from the work we report here is that the cochlear apex of people exposed to such low-frequency sounds will be stimulated to a far greater degree than is suggested by their measured A-weighted sound level. The demonstration that sounds in the range of 40 – 45 dB A may be causing intense stimulation of the cochlear apex has not previously been appreciated. This may account for why the influence of low frequency noise on humans is greater than that estimated from spectral measurements and why consideration of noise crest factors is appropriate²⁰. The fact that apical stimulation is maximal when mid- and high-frequency components are absent from the sound may also be important to wind turbine noise effects. It is known that people's houses attenuate sound frequencies in the audible range but have little influence, or may even increase infrasound and low-frequency sound levels²¹. Thus, prolonged periods of exposure to wind turbine noise in an otherwise quiet environment (such as a quiet bedroom) seems to represent a condition in which apical stimulation would be maximized. Intense stimulation of the cochlear apex will certainly have some influence on human physiology. On this basis we think that the concept of

“what you can’t hear can’t hurt you” is false. Similarly, there are potential mechanisms by which low-frequency sounds could influence vestibular physiology which are being ignored by some²². Our measurements showing that the ear generates large electrical responses to low-frequency stimulation suggest that the effects of low-frequency sound on people living near wind turbines should not be dismissed by those with little understanding of how low frequency sounds indeed affect the ear^{19,21,22}. More research on this topic is necessary to enlighten the scientific, medical, and legal communities, and the public, some of whom are being chronically exposed to these sounds.

5 ACKNOWLEDGEMENTS

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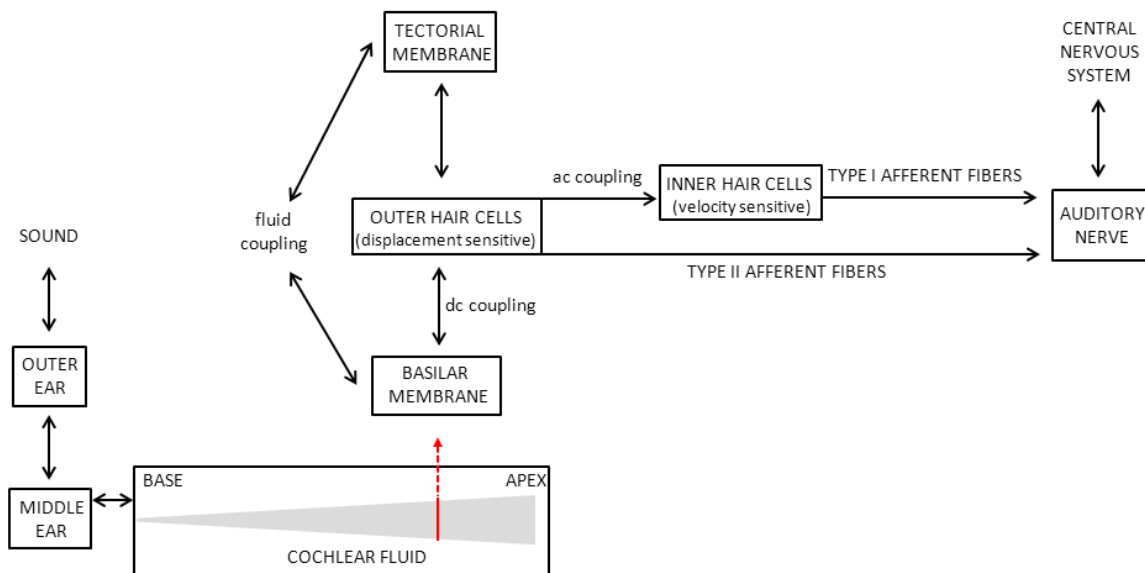


Fig. 1 – Schematic of the auditory periphery. The guinea pig cochlea has 1 row of inner hair cells (IHC) and three rows of outer hair cells (OHC) along its length. The red line and subsequent compartments describe some anatomical and physiological properties of a given segment. After sound propagates through the outer and middle ear, the basilar membrane is set into motion. OHCs in the cochlear apex region respond to low frequencies and are sensitive to the displacement of the basilar membrane motion and are dc-coupled to the sound stimulus. In contrast, the IHC are free within the cochlear fluid causing them to be excited by the velocity of basilar membrane motion, are ac-coupled to the sound stimulus, and are insensitive to low frequency stimulation.

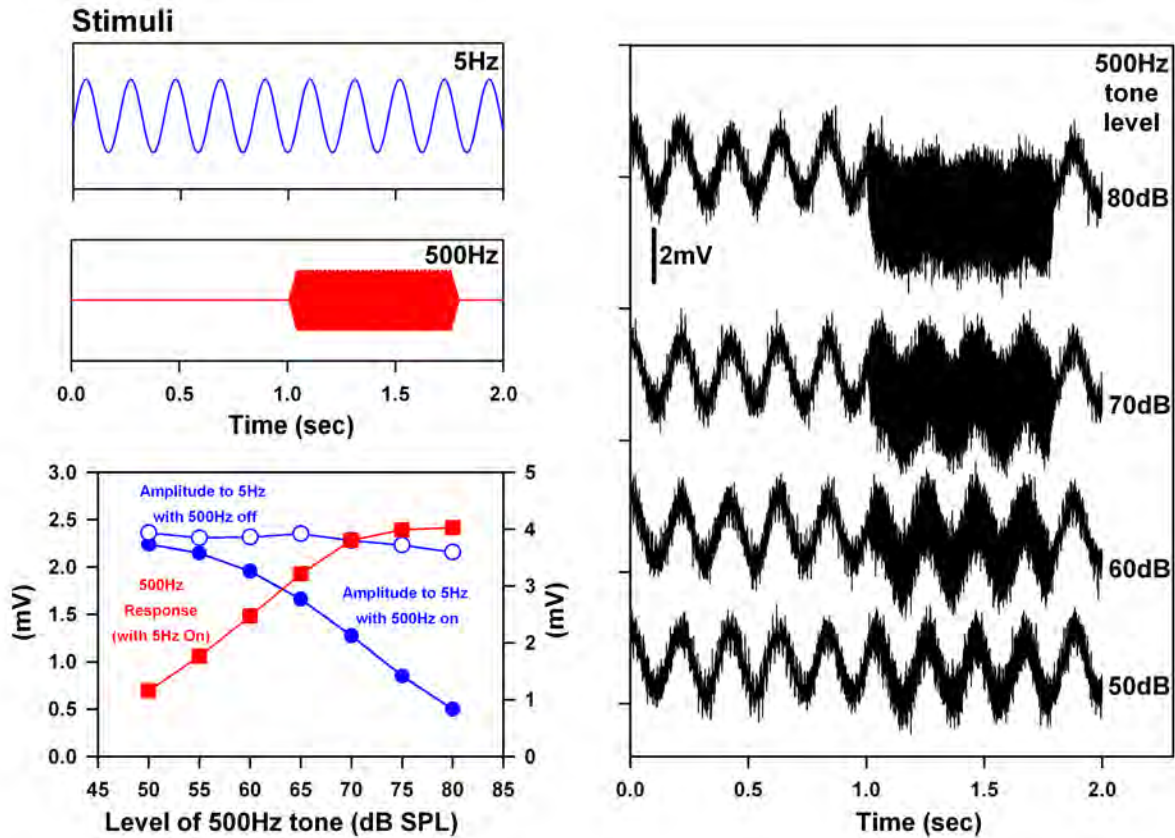


Fig. 2 – Higher-frequency stimuli (500 Hz) suppress the response to a very low-frequency (5 Hz) stimulus. A 500 Hz tone of varied level was superimposed on a 5 Hz, 90 dB stimulus. As the level of the 5 Hz tone was increased, the amplitude of the 5 Hz response declined (right panel). Amplitude measurements (lower left panel) show that 5 Hz stimulation well below saturation (red curve) caused a reduction in response amplitude to the 5 Hz.

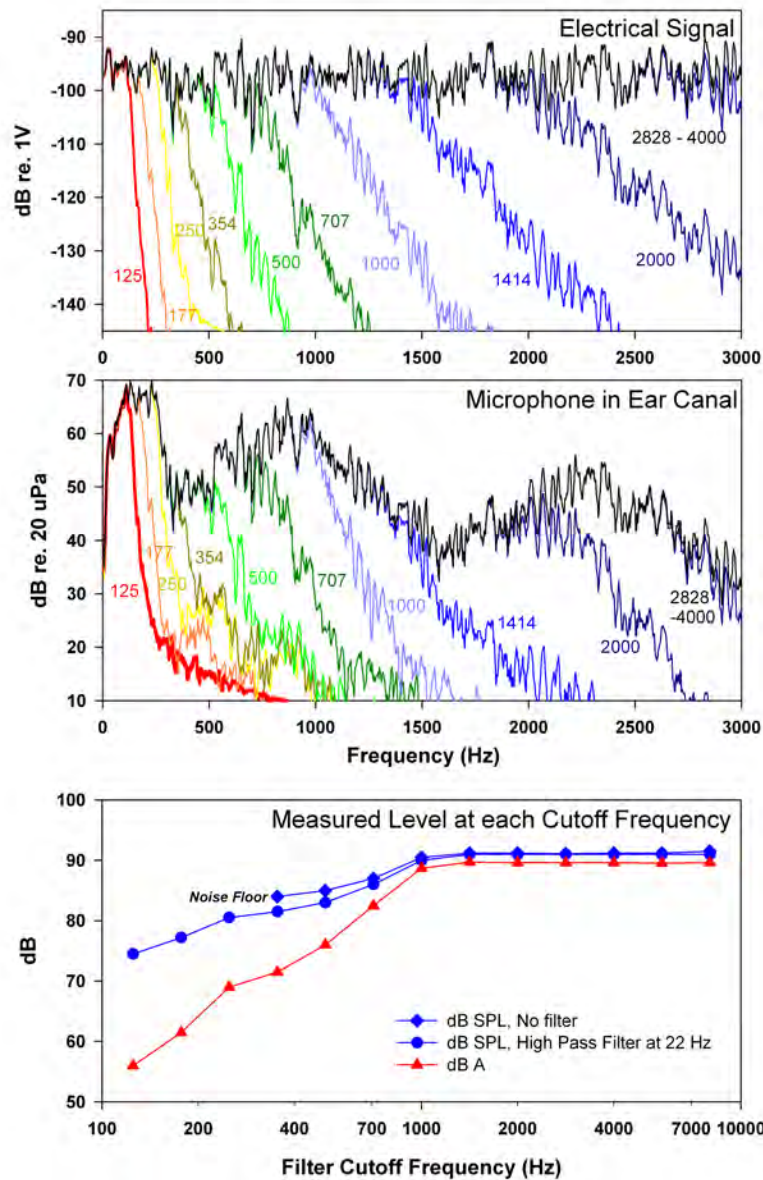


Fig. 3 – Low-pass noise used in this study. The upper panel shows the spectra of low-pass noises with cutoff frequency varied from 125 Hz to 4 kHz. Note that the filter altered the high-frequency component of the noise but had no influence on the low-frequency content (below 100 Hz). The cutoff slope was approximately 55 dB/octave. The middle panel shows the stimuli measured in vivo in the external canal after being delivered by the Sennheiser headphone. The lower panel shows the stimuli with different cutoff frequencies measured in dB SPL and in dB A.

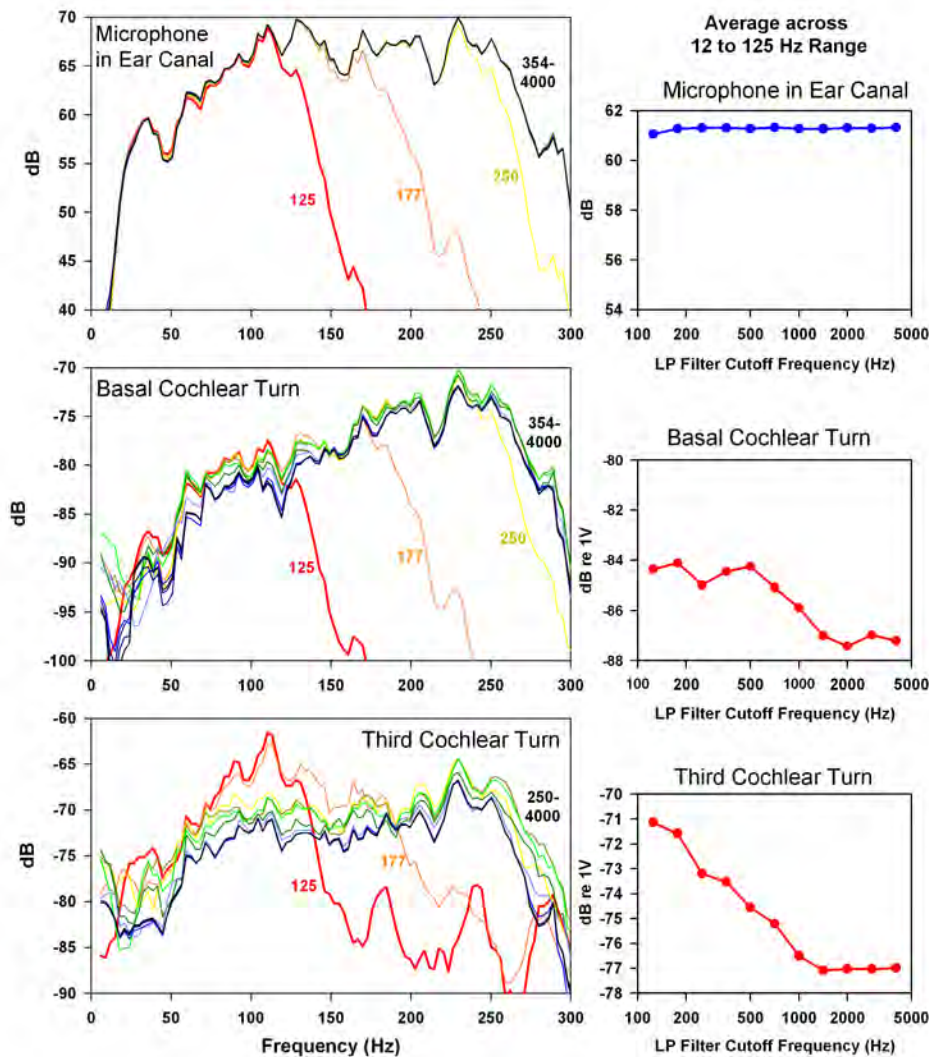


Fig. 4 – Spectra expanded to show the lowest 300 Hz of the frequency range. At each noise filter cutoff frequency, all responses (all 3 panels) were recorded simultaneously to the same stimuli. Top row: The low-frequency region of the sound field showed little variation as cutoff frequency was changed. Middle Row: Responses from the basal cochlear turn were larger when high frequency components were absent. Bottom Row: Apical (Third turn) responses were substantially greater when high frequency components were absent. In this case, the lowest band of noise (125 Hz cutoff) generated ~ 6 dB larger responses than the widest band of noise (4 kHz cutoff). For each condition, the average spectral level in to 12 Hz to 125 Hz range was graphed relative to the noise filter cutoff frequency in the right column.

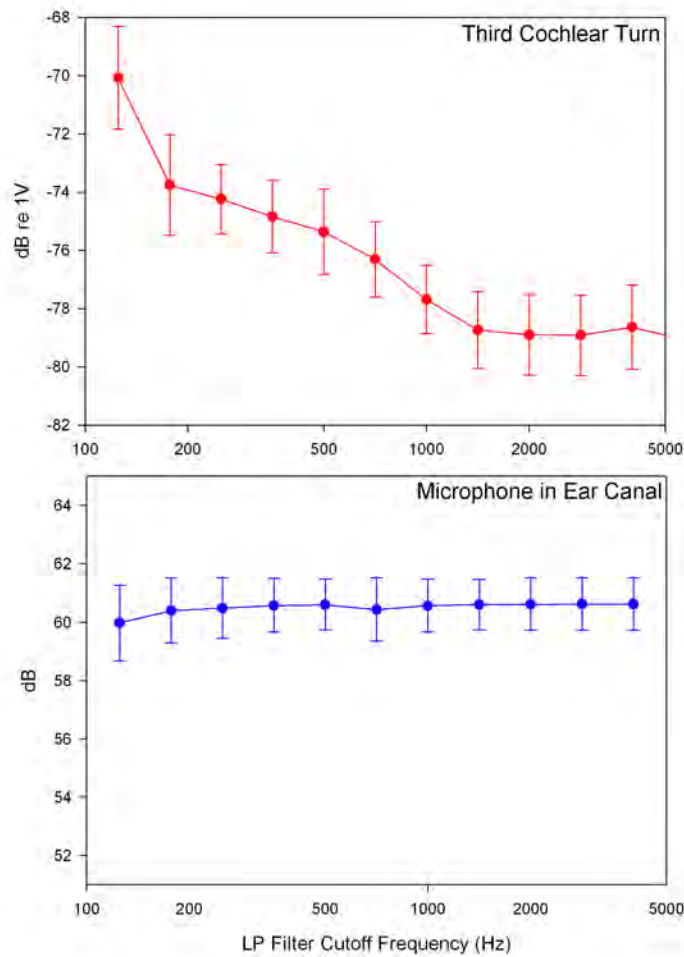


Fig. 5 – Average response amplitudes (+/- SD) in 5 experiments. At each noise cutoff frequency, response amplitude was measured as the average spectral level in the 12 – 125 Hz range. Responses from the apical region of the cochlea showed a systematic decline as noise cutoff frequency is varied, while responses from the microphone, analyzed in an identical manner, did not.

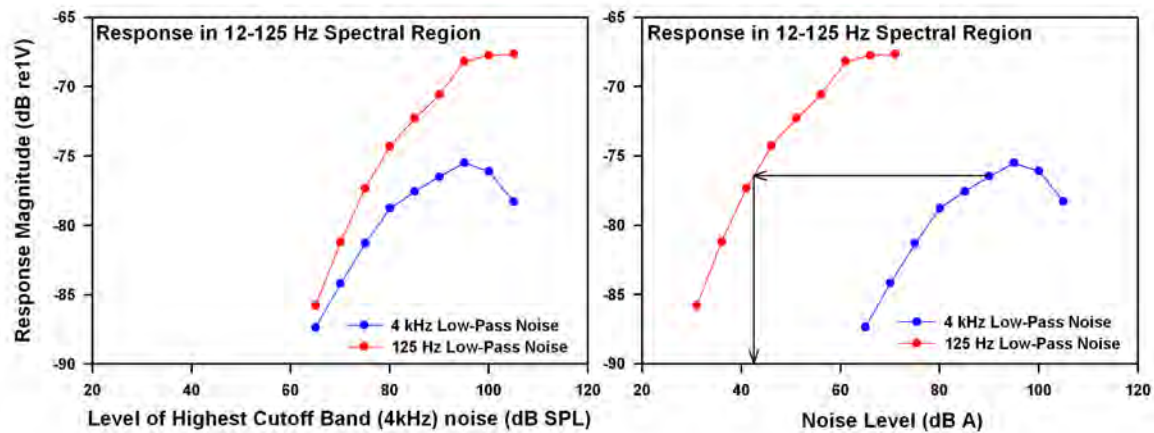


Fig. 6 – **Left panel:** Response amplitudes as the noise level was varied in 5 dB steps. Shown here is only the response amplitudes to the lowest (125 Hz) and highest (4 kHz) noise filter cutoff frequencies used. Amplitudes were the average from the spectrum across the 12 – 125 Hz range (as in Figure 4). Noise with higher frequencies present always generated lower response amplitudes than when higher frequencies were absent. For the 4 kHz band, the responses saturate and decline as level was increased, while the responses to the low-band (125 Hz cutoff) noise keep increasing. **Right panel:** The same data plotted based on the A-weighted level of the stimuli measured at each cutoff frequency. This shows that low-frequency noise (125 Hz cutoff) of ~43 dB A generated as large of a response at low frequencies as did a 90 dB A wide band (4 kHz cutoff) noise. Indeed, for 125Hz low-pass noise of 45 dB A or greater, an ear's response will be larger than for wide band noise presented at ANY level.