Published in final edited form as:

Ear Hear. 2013 January ; 34(1): 42–51. doi:10.1097/AUD.0b013e31825f9bd3.

A new auditory threshold estimation technique for low frequencies: Proof of concept

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Abstract

Objectives—Presently available non-behavioral methods to estimate auditory thresholds perform less well at frequencies below 1 kHz than at 1 kHz and above. For many uses, such as providing accurate infant hearing aid amplification for low-frequency vowels, we need an accurate non-behavioral method to estimate low-frequency thresholds. Here we develop a novel technique to estimate low-frequency cochlear thresholds based on the use of a previously-reported waveform. We determine how well the method works by comparing the resulting thresholds to thresholds from onset-response compound action potentials (CAPs) and single auditory-nerve (AN) fibers in cats. A long-term goal is to translate this technique for use in humans.

Design—An electrode near the cochlea records a combination of cochlear microphonic (CM) and neural responses. In response to low-frequency, near threshold-level tones, the CM is almost sinusoidal while the neural responses occur preferentially at one phase of the tone. If the tone is presented again but with its polarity reversed, the neural response keeps the same shape, but shifts ½ cycle in time. Averaging responses to tones presented separately at opposite polarities overlaps and interleaves the neural responses and yields a waveform in which the CM is cancelled and the neural response appears twice each tone cycle, i.e. the resulting neural response is mostly at twice the tone frequency. We call the resultant waveform “the auditory nerve overlapped waveform” (ANOW). ANOW level functions were measured in anesthetized cats from 10 to 80 dB SPL in 10 dB steps using tones between 0.3 and 1 kHz. As a response metric, we calculated the magnitude of the ANOW component at twice the tone frequency (ANOW2f). The ANOW threshold was the sound level where the interpolated ANOW2f crossed a statistical criterion that was higher than 95% of the noise floor distribution. ANOW thresholds were compared to onset-CAP thresholds from the same recordings and single-AN-fiber thresholds from the same animals.

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Conflicts of Interest:

For NPC, none are declared.

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Results—We obtained ANOW and onset-CAP level functions for 0.3 to 1 kHz tones, and single-AN-fiber responses from cats. Except at 1 kHz, typical ANOW thresholds were mostly 10-20 dB *more* sensitive than onset-CAP thresholds and 10-20 dB *less* sensitive than the most sensitive single-AN-fiber thresholds.

Conclusions—ANOW provides frequency-specific estimates of cochlear neural thresholds over a frequency range that is important for hearing but is not well accessed by non-behavioral, non-invasive methods. Our results suggest that, with further targeted development, the ANOW low-frequency threshold estimation technique can be useful both clinically in humans and in basic-science animal experiments.

Keywords

audiogram; auditory nerve neurophonic; compound action potential; neural synchrony; phase locking

INTRODUCTION

Accurate non-behavioral estimates of low-frequency auditory threshold have classically been difficult to obtain. Presently available threshold estimation techniques based on distortion-product otoacoustic emissions, compound action potentials (CAPs), auditory brainstem responses (ABRs), and auditory steady-state responses perform well at high-frequencies (e.g., Schoonhoven et al. 1996; Sininger et al. 1997; Aoyagi et al. 1999; Rogers et al. 2010). Unfortunately, these techniques perform less well at low-frequencies, i.e., below 1 kHz or so (Spoor and Eggermont 1976; Gorga et al. 1993; Picton 2007; Sininger 2007). For instance, compared to behavioral pure-tone thresholds, the thresholds at 500 Hz from electrocochleography show a wider spread of threshold differences and a slope further from unity than electrocochleography thresholds at 1, 2 or 4 kHz (Spoor and Eggermont 1976).

A technique to estimate low-frequency cochlear thresholds would provide an important complement to the established high-frequency threshold estimation techniques in the clinic. For example, accurate infant hearing aid amplification for low-frequency vowels is certainly as important as amplification for higher frequency speech and environmental sounds. The Joint Committee on Infant Hearing (2007) recommends that management of permanent hearing loss begin no later than six months of age to significantly reduce the chance of delays in speech and language development (e.g., Moeller 2000). Since it is time consuming and not always possible to obtain reliable behavioral threshold measures from infants, assessment guidelines state that the most appropriate strategy is to use frequency-specific ABR (ASHA 2004). Low-frequency tone pips do not evoke useful early ABR peaks of cochlear origin (e.g., Johnstone et al. 1979, Sininger 2007 Figure 12.7). The use of the mid-brain generated ABR Wave 5 or auditory steady-state responses for threshold estimation requires the use of a correction factor that varies across subjects and adds additional uncertainty (Stapells 2000; Cone-Wesson and Dimitrijevic 2009). Clinicians are thus left with no non-behavioral measure of direct cochlear origin that accurately estimates low-frequency cochlear thresholds.

Here we take a recording process that has been described before (Eggermont 1974; Ferraro et al. 1994; Henry 1995) but which has not been usefully quantified, and demonstrate that it can be used to estimate low-frequency cochlear thresholds. To apply this technique, we used recordings of cat round window (RW) potentials in response to tones, but ear-canal or tympanic membrane recordings could be used in humans. Cochlear responses from an electrode on, or near, the RW include contributions from extracellular potentials generated

by hair cells and auditory-nerve (AN) responses. The largest and most important extracellular potential generated by hair cells is the cochlear microphonic (CM) potential which is predominantly from outer hair cells and nearly sinusoidal for low-level tones (Dallos and Cheatham 1976). AN fibers with low characteristic frequencies (CFs) respond to low-level pure tones in a phase-synchronized fashion, i.e., they fire preferentially during one phase of the stimulus tone. This “phase locking” results from the oscillating receptor potentials of inner hair cells preferentially evoking AN discharges during their depolarization phase (Palmer and Russell 1986). Since, near threshold, low-CF AN fibers have phase-locked responses at predominately one stimulus phase, the AN response from low-level, low-frequency tones is partially half-wave rectified. Thus, in response to low-level, low-frequency tones, the potentials recorded with an electrode near the cochlea contain a nearly-sinusoidal CM and a partially half-wave-rectified AN response.

Our goal is to obtain an estimate of the neural thresholds at low frequencies from a cochlear response that is a mix of CM + AN responses. Our technique starts with a commonly-used technique to cancel CM, namely alternating the polarity of a tonal stimulus and averaging the resulting responses. Since the CM reverses when the tone reverses, averaging RW responses from pure-tone stimuli of opposite polarities cancels the CM. In contrast, when the tone is presented with the opposite polarity, the AN response waveform shape stays the same but, because of the half-wave rectification, the AN response moves one half cycle in time. Averaging responses to tones with opposite phases interleaves the partially rectified AN response waveforms in time. The waveform that remains after averaging therefore results primarily from cochlear neural responses and is dominated by spectral components at twice the tone frequency (see Henry 1995 for an experimental confirmation of this). We use this potential to measure AN low-frequency thresholds.

We refer to the waveform obtained after opposite-polarity tone responses are averaged as the “auditory nerve overlapped waveform” (ANOW). We say “overlapped” because the neural potentials occur at different times (relative to stimulus onset) so their peak values do not add when averaged, but instead cause the resulting neural response waveform to be at twice the tone frequency. ANOW is a CAP in the sense that it results from the compounding of responses from many single-AN-fibers. However, we will use the term “CAP” only as it is commonly used: recordings of many single-AN-fiber responses that are synchronized to the transient onset (or offset) of a sound. In contrast, ANOW is produced by many single-AN-fibers that are responding during ongoing tones.

In this paper we describe our initial methods for ANOW measurements and compare ANOW thresholds to onset CAP thresholds and single-AN-fiber thresholds recorded from the same ears. We do not claim to have optimized the technique for obtaining ANOW thresholds or to be providing a definitive statement of the difference between ANOW thresholds and single-AN-fiber (or behavioral) thresholds. Achieving these goals will require further work with the ANOW electrode in the place to be used in the species of interest. The result presented here, together with the work of Henry (1995), is a proof of the concept that ANOW thresholds can be used to estimate AN thresholds.

METHODS

Overview

Several steps are needed to quantify ANOW thresholds. First, we presented tones at different frequencies, and at each frequency presented a series of levels with alternating tone polarities. Then at each frequency and level we averaged the responses from both polarities of tones and calculated ANOW magnitudes. Finally, we calculated thresholds from the level functions of ANOW magnitude at each frequency

The results reported here were obtained from animals in which recordings from single-AN-fibers were made in combination with other experiments (e.g. Berezina and Guinan 2011; Nam and Guinan 2011; Lichtenhan et al. unpublished). Using these animals enabled us to compare the ANOW thresholds with a large variety of single-AN-fiber thresholds. However, many things such as the placement of the electrodes and the timing of the measurements were controlled by the needs of these other experiments. Since the cat preparation in our laboratory has been fully described before (Kiang 1965; Stankovic and Guinan 1999; Guinan et al. 2005), we give only a brief outline below. Of greater importance is to describe the methods involved in ANOW stimulus and analysis.

Stimulus selection, presentation and ANOW recordings

Since the ANOW technique depends on cancelling the CM in the response, we presented relatively short tone bursts at a high repetition rate, with alternate tone bursts being reversed in polarity. We avoided averaging responses from positive tone bursts and then separately averaging responses from negative tone bursts because if the preparation changed between one recording and the next, then the CM from the two recordings might not be equal in amplitude and cancel in the average. We used a repetition period of 202 ms with tone bursts of opposite polarity starting one half repetition period apart so that the time between bursts was constant. The repetition period was not commensurate with the 60 Hz power frequency to reduce 60 Hz interference in the average. Typically, responses from 64 repetition periods were averaged. Each tone burst was 30 ms in duration from the onset to offset half-amplitude points. To reduce frequency splatter in the stimulus, we used a raised-cosine-ramp onset/offset that transitioned from 0 to unity over two periods of the tone frequency. For 300 Hz, our lowest frequency, this ramp lasted 6.67 ms. The tone-burst frequencies were 300, 400, 500, 700 and 1,000 Hz.

Stimulus calibration, stimulus generation, and data acquisition were done with data-acquisition boards (National Instruments: PXI 4461 24 bit Dynamic Signal Analyzer and PXI 6221 16 bit M-series) in a PXI chassis controlled by LabVIEW custom-written software. ANOW stimulus presentation and measurements used a 100 kHz sampling frequency. Ear canal acoustics were measured with an electret microphone (Knowles EK-23028 or 23133). Stimuli were delivered with a 1 inch condenser earphone (Bruel & Kjaer 4145). The microphone and earphone were housed in a custom acoustic assembly (Kiang 1965). ANOWs were recorded with a silver wire placed on or near the cochlea or on the RW (see Results) and were amplified 80 dB and filtered from 100 Hz to 10 kHz by a GRASS amplifier (Astro-Med, Inc. GRASS Instrument Division, model P511). For display in Figures 2 and 3, the ANOWs were additionally digitally filtered 0.1-5 kHz (MATLAB's `filtfilt` function).

Analysis

To cancel the CM and obtain ANOWs, responses from stimuli with positive and negative polarity were averaged together. From the resulting ANOW, we wanted to extract the component at twice the tone frequency while excluding the initial part that contained the onset CAP. A 20 ms analysis window was used so that there were an integral number of cycles at our tone frequencies, all of which were multiples of 50 Hz. Since the longest tone-burst onset ramp was 6.67 ms and there is ~1 ms neural delay, the response included in the analysis was the window starting 7.67 ms after the beginning of the tone onset ramp. To reduce frequency splatter in the analysis, we applied ramped edges (a raised cosine that transitioned from 0 to unity over 2 periods of the tone being analyzed) to the 20 ms analysis window and normalized the resulting window so that its average amplitude was unity. The averaged data from 7.6 to 27.67 ms after the start, multiplied by the ramped window, was transformed to the frequency domain by a fast Fourier transform (FFT). For each tone

frequency and level, the ANOW magnitude at twice the tone frequency (ANOW2f) was determined from the spectral bin at twice the tone frequency.

ANOW threshold for each frequency was taken to be the sound level at which the ANOW2f magnitude versus tone level crossed a criterion value. A fixed electrode position and fixed magnitude criterion would be expected to yield the most consistent threshold determination. However, our tests in different animals had varying electrode positions so we took the ANOW threshold to be the level at which the ANOW rose above criteria set by the measurement noise floor. To estimate this noise floor, we did exactly the same analysis as was done to obtain ANOW on two 20 ms windows that did not overlap the tone response or each other (i.e. they only contained the background response noise). A separate analysis was done at each ANOW frequency on all of the waveforms obtained in a set ($N = 80 = 5 \text{ frequencies} \times 8 \text{ sound levels} \times 2 \text{ windows}$). FFT magnitudes calculated from random noise form a Raleigh distribution (Backus, 2007), so the magnitudes we obtained were fit by the MATLAB “raylfit” function. For each ANOW frequency, the threshold criterion was set to at a level that was greater than 95% of the points in the noise distribution. This is equivalent to the ANOW at threshold achieving statistical significance at the $p=0.05$ level. All analyses were performed with custom written software in MATLAB.

For comparison with the ANOW thresholds, onset-CAP thresholds were calculated by a similar method. Starting from the same waveform averages of the responses from stimuli with positive and negative polarity, we selected a 10 ms window that started 1 ms (to compensate for neural delay) after the foot of the tone onset, and we took the CAP amplitude to be the peak-to-peak (p-p) value in this window. To obtain noise measurements, comparable p-p measurements were made on each waveform from five 10 ms windows that did not overlap the tone response or each other. This was done for every waveform in a set yielding 200 noise measurements ($5 \text{ frequencies} \times 8 \text{ sound levels} \times 5 \text{ windows}$). A Kolmogorov-Smirnov test showed that six of the seven data sets used in this paper were well fit ($p < 0.05$) by a normal distribution and the seventh was not far off. All of the distributions were fit by a normal distribution and the onset-CAP threshold criterion was set to level that was greater than 95% of the points in the p-p noise distribution.

Throughout the measurements of single-AN-fiber responses, our standard onset-CAP measurement paradigm was used at frequencies 2 kHz and above, to determine if auditory sensitivity changed. This used responses from the same electrode that recorded the ANOW responses. Our standard onset-CAP method used an up-down adaptive procedure that sought the sound level that produced a 10 μV p-p response from short averages of alternating-polarity 5 ms tone pips.

Animal Procedures

ANOW, onset-CAP, and single-AN-fiber recordings were performed in cats anesthetized with pentobarbital and urethane. All other surgical and single-AN-fiber recording procedures were done as previously described (Kiang 1965; Stankovic and Guinan 1999; Guinan et al. 2005). The left ear was used for all experiments. The bulla was opened and a silver wire electrode was placed on the bone near the cochlea. At the end of some experiments, the electrode was moved directly onto the RW. General cochlear health was monitored throughout the experiment with CAP-audiograms from the silver wire electrode and click-evoked ABRs from a screw electrode at the vertex. After making contact with a single-AN-fiber with an electrolyte-filled pulled-pipette electrode, a tuning curve was obtained by an automated procedure. The fiber threshold was obtained from the most sensitive frequency of a quadratic fit to the tuning curve points that were within 10 dB of the most sensitive point of the unprocessed tuning curve. Animals were euthanized at the

conclusion of the experiment. All procedures were approved by the Institutional Animal Care and Use Committee at the Massachusetts Eye and Ear Infirmary.

RESULTS

ANOW Properties

Examples of non-overlapped responses from tones of opposite phases are shown in Figure 1A and B. These cochlear responses contain both CM and AN responses but are dominated by CM generated by outer hair cells. CM domination is illustrated by the reversal of response polarity (see the vertical line marking the same time in panels A and B). The negative peaks at the beginning of these responses that are most easily seen in panel B are due to onset CAPs superimposed on the CM.

The waveform derived from averaging the responses of Figure 1A and B is shown in Figure 1C. The amplitude of the resulting waveform is considerably lower than the original waveforms because the CM is cancelled. The waveform of panel C shows two main features. First, there are two negative deflections near the beginning of the response that are mostly the averaged onset CAPs seen in panels A and B. These deflections may also contain some un-cancelled summing potential (the summing potential is not seen throughout the response because of high-pass filtering). The second and most important feature is that throughout the waveform there is an oscillation at twice the frequency of the tone (and twice the frequency of the CM-dominated cochlear response of panels A and B). *This is the ANOW.* The ANOW is smaller than the cochlear response potentials (note the difference in amplitude scales from panels A and B to panel C). An ANOW at twice the tone-burst frequency is consistent with the expectation that ANOW comes from averaging, and thus overlapping, two partially half-wave rectified neural responses with one shifted one half cycle in time relative to the other.

Examples of ANOWs from different sound levels are shown in Figure 2. In the data illustrated, the ANOW threshold was between 20 and 30 dB SPL. Both the ANOW and the onset CAP can be seen to increase as sound levels increased above threshold. In these examples and most others, the ANOW phase changed little with level. In some cases at mid-levels, the ANOW phase shifted dramatically, and at a sound level in the middle of the shift, two peaks could be seen during each twice-the-tone-frequency cycle. This phenomenon has been called “peak splitting” and has been previously shown in cochlear, hair cell, ANOW, and single-AN-fiber responses (Snyder and Schreiner, 1984; Kiang 1990; Henry 1995; Cheatham et al. 1998).

The ANOW amplitude decreased from its onset to later in the response, i.e. it showed adaptation. ANOW adaptation can be most clearly seen at high sound levels (Fig. 2, left). As expected, CM-dominated cochlear response waveforms (e.g. Figure 1A and B) showed little adaptation, and the little adaptation that was present can be attributed to small neural responses adding to the CM.

ANOWs from different tone-burst frequencies at a fixed sound level are shown in Figure 3, left. Figure 3, right, is an expanded view showing one cycle at the tone frequency with the ANOW averaged, in one-cycle blocks, over the time indicated by the horizontal bar on the left. At 300 Hz, the ANOWs sometimes appeared to be short (~1/2 ms) pulses of neural response that occurred twice each tone-frequency cycle (e.g. Fig. 3, bottom right) and at other times the waveforms were more complex. As tone frequency increased, ANOWs became more sinusoidal. At the highest frequencies, ANOWs typically looked almost sinusoidal with oscillations twice each tone-frequency cycle (Fig. 3, top right). The smoothly varying, near-sinusoidal shape of the high-frequency ANOW can be thought of as

resulting from the response pulses seen at 300 Hz being sharpened because the underlying single-AN-fiber unitary potentials are more tightly grouped in time due to the tone cycle being shorter, with any sharp edges being removed by our filtering.

One interesting phenomenon that can be seen in the 500 Hz response of Figure 3 is a small offset response around 34 ms. Offset responses were occasionally seen and are presumably due to “frequency splatter” exciting AN fibers at frequencies near the tone frequency which have not been adapted by the ongoing tone and therefore respond well to the splatter (Henry & Lewis 1988).

Measuring ANOW amplitude

To derive a metric for the amplitude of the neural responses represented in the ANOWs, we first had to select which part of the waveform to be included in our analysis window. We used 20 ms of the response starting 7.6 ms after the beginning of the tone-burst ramp, as shown by the bar below 1000 Hz in Figure 3. This delayed start avoided the most rapid part of the adaptation but did not guarantee that the ANOW during the window was in a steady state (e.g. Figs. 2, 3). Since the ANOWs were adapting during the analysis window (particularly at high levels), their spectra had energy at frequencies near, as well as at, multiples of the tone-frequency. To reduce the effect of such frequency splatter in the analysis, we used edge smoothing on the analysis window (Methods).

The ANOW selected by the edge-smoothed window was transformed to the frequency domain using an FFT. This FFT effectively averaged the response over all of the cycles in the 20 ms window. Example ANOWs in the frequency domain from the five tone frequencies are shown in Figure 4. Notice the near absence of spectral energy at the tone frequency. This reflects the almost perfect cancellation of the CM.

The ANOW frequency-response structure depended on the tone frequency (Fig. 4). ANOWs evoked by tone frequencies of 500 Hz and above typically had a single large spectral peak at twice the tone frequency with little energy at other frequencies. In contrast, tones at 400 Hz and below showed significant energy at higher multiples of the tone frequency, with the relative amount of energy at the higher multiples increasing as tone frequency decreased (e.g. Fig. 4). This frequency-response pattern across tone frequency can be understood from the ANOWs shown in Figure 3 – particularly in the expanded view of ANOW averaged over one tone-frequency cycle. At low frequencies, the ANOW appears to be more like a pulse of a fixed duration than a sinusoid. A fixed pulse duration that is shorter than half the original-tone period can produce the frequency-response pattern seen in Figure 4, top. The fixed duration of the pulsed response is what might be expected if the nerve fibers fire at approximately the same phase on each cycle, and the durations of single-AN-fiber unitary responses are similar (0.3-0.5 ms wide) for low-CF fibers as for high-CF fibers (Kiang et al. 1976; Prijs 1986).

Since the ANOW frequency-response structure can be complex, for simplicity we chose the ANOW magnitude to be the amplitude of the component at twice the tone frequency. Choosing a more complicated metric, e.g. some combination of the components at two and four times the tone frequency, would make the ANOW magnitudes larger at tone frequencies 400 Hz and below. Whether this would change the ANOW threshold would depend on how a corresponding noise level is computed. ANOW amplitudes as a function of tone level for all of the frequencies tested on one animal are shown in Figure 5A. For comparison, the magnitudes of the un-cancelled waveforms – the cochlear responses from which the ANOWs were derived – are shown in Figure 5B. In general, the growth of the cochlear response was more linear than the growth of the ANOW, but both can show nonlinearities.

Determining ANOW threshold

At each frequency, ANOW threshold was determined as the sound level at which the ANOW2f magnitude first exceeded the noise floor criterion. The criterion was chosen to be the lowest value that was significantly above the noise at $p < 0.05$. This threshold criterion provided a kind of self-normalization for differences in electrode placement. The ANOW amplitude was larger for an electrode on the RW than for an electrode far from the RW. However, the noise level was also larger when the electrode was on the RW – a result that is consistent with the interpretation that most of the noise was due to what is called “RW noise” (Dolan et al. 1990). An alternative could be to use a fixed level as the ANOW criterion. The best type of ANOW criterion likely depends on where the electrode is positioned. It is clear from Figure 5 that small changes in the criterion can produce substantial changes in the resulting ANOW thresholds.

Comparison of ANOW thresholds with single-AN-fiber and onset-CAP thresholds

To determine how well ANOW thresholds compare to neural thresholds, we made ANOW measurements during experiments in which the thresholds of single-AN-fibers were measured. Since the single-AN-fiber measurements were made in combination with other experiments, the ANOW electrode was originally put at a location determined by the other experiment and the distance from the RW varied across experiments. In all cases the ANOW electrode was used to obtain our standard onset CAP measurements, at frequencies 2 kHz and above, throughout the experiment to monitor the sensitivity and stability of the ear. Here we use data only from experiments in which the standard onset CAP thresholds remained stable within a range of 10 dB. In three experiments, after the single-AN-fiber recordings were done, the electrode was moved directly onto the RW membrane and ANOW recordings were made. In one of these experiments and four others, ANOW recordings were made with the electrode on, or near, the cochlea but not directly on the RW. Results from the three experiments with the electrode placed directly on the RW and from three other experiments with a non-RW electrode are shown in Figure 6 (only the low-frequency region is shown in Fig. 6, center and right, because these experiments focused on fibers below 2 kHz and obtained few fibers above 2 kHz).

The relationship between ANOW thresholds and single-AN-fiber thresholds depended on tone frequency and electrode position. At 300-700 Hz, ANOW thresholds were approximately 10-20 dB above the thresholds of the most sensitive (lowest threshold) AN fibers (Fig. 6). In this frequency region, the single-AN-fiber thresholds typically increased as tone frequency decreased and the ANOW thresholds followed this trend. ANOW measurements from electrodes placed directly on the RW were more consistent than the non-RW positions, although there are not enough data to make a strong statement on this. For 1000 Hz tones and off-RW electrodes, the ANOW thresholds were often more than 20 dB above the most sensitive single-AN-fiber frequencies. The single-AN-fiber responses were generally more sensitive at 1000 Hz than at 700 Hz, but at all four of the non-RW electrode positions in Figure 6, ANOW thresholds were less sensitive at 1000 Hz than at 700 Hz. In contrast, at all three of the RW electrode positions, ANOW thresholds were more sensitive at 1000 Hz than at 700 Hz.

For 300-700 Hz tones, most ANOW thresholds were 10-20 dB more sensitive than onset CAP thresholds (Fig. 7). For 1000 Hz tones, ANOW lost its advantage over onset CAP. For all three cases with the electrode on the RW, ANOW was 5-10 dB more sensitive than onset CAPs but for three of four cases with the electrode not on the RW, CAP was 10-15 dB more sensitive than ANOW. Finally it should be noted that the standard onset CAPs shown in Figure 6 at 2000 Hz and above were done by a different method than that used for ANOW,

so these onset CAPs should not be considered as an extension of those at 1000 Hz and below.

DISCUSSION

ANOWs provide a new way to estimate low-frequency cochlear thresholds. In this section we first consider several questions relating to how well ANOWs work, and we evaluate the frequency and level ranges over which ANOW thresholds are useful. We then review the history of ANOW-like methodology. We conclude by describing clinical and basic-science circumstances in which ANOW measurements may be helpful.

Is the ANOW really a neural response?

The evidence that ANOWs are a neural response depends heavily on the work of Henry (1995) and the fact that our ANOW is essentially the same as Henry's "auditory-nerve neurophonic (ANN) residual". First, as noted by Henry, reversing the polarity of the tone stimulus and adding the resulting responses does an excellent job of cancelling CM. We confirmed this by finding that the energy at the tone frequency in ANOW is negligible (Fig. 4), which indicates near perfect cancellation of CM, at least at its first harmonic. A second line of evidence is that ANOW shows adaptation (Figs. 1-3), particularly at higher levels, which is a characteristic of neural potentials but not hair-cell potentials. Finally, the application of TTX, a chemical that blocks neural responses, greatly reduced "ANN residuals" (i.e., ANOWs), but, in contrast, did not reduce CM (Henry 1995). This evidence makes a compelling case that ANOWs are due to neural potentials.

Do ANOW thresholds represent the thresholds of fibers tuned to the tone frequency?

It is important to consider if ANOW is generated by cochlear neurons with CFs at the tone frequency. The most drastic other possibility is that ANOW might be due to high-CF neurons responding to low-frequency sounds, i.e. high-CF neurons responding in the low-frequency "tails" of their tuning curves. High-CF fibers in experimental animals have tail-frequency thresholds of 60-70 dB SPL near 1 kHz and somewhat higher thresholds at lower frequencies (Kiang and Moxon 1974; Patuzzi et al. 1988; Temchin et al. 2008). There are no good data from humans on tail thresholds for high-CF AN fibers. Because of this, any ANOW evoked from 60-70 dB SPL or higher should be considered as possibly being generated, at least in part, by high-CF fibers. This may put an upper limit on the extent to which pathologic low-frequency thresholds can be assessed with ANOW methods in the presence normal high-frequency thresholds. Even cases with pathologic high-frequency thresholds must be looked at very critically because acoustic trauma can greatly raise tip thresholds of high-CF fibers while simultaneously lowering their tail thresholds (Liberman and Dodds 1984). Perhaps continuous high-pass noise that adapts the high-CF fibers would reduce potential interference from these fibers.

Another consideration is how well ANOW represents responses from fibers with CFs at the tone frequency versus fibers with nearby CFs. As the tone level is increased, fibers with CFs further from the tone frequency are excited and contribute to ANOW. This is a greater problem at low frequencies than at high frequencies because tuning curves are wider (in octaves) at low frequencies than at high frequencies. Measurements that exclude the response to the onset transient (as the ANOW method does) avoid the frequency splatter due to stimulus onset. In contrast, onset CAPs and ABRs are influenced by such frequency splatter and at low frequencies in particular, this reduces their frequency specificity. Overall, an ANOW that excludes the onset response provides a metric that is more focused in terms of fiber CF than onset-response metrics, at least for levels below 60-70 dB SPL.

Yet another consideration is that ANOWs might include responses from brainstem neurons (i.e. from ABR responses). Kainic acid injections into the cochlear nucleus, which obliterated its neurons, had little effect on “ANN residuals” (i.e. ANOWs) (Henry 1995). While our ANOWs do not show any obvious ABR (e.g., Figure 1), some contamination of ANOW by ABR cannot be ruled out. However, it seems unlikely that there is a significant brainstem contribution to ANOW.

Neural Synchrony, CAP, ANOW and single-AN-fiber unitary potentials

Since ANOW depends on neural synchrony, it is useful to consider the multiple uses of the term “neural synchrony.” Neural synchrony has been used to denote both “onset synchrony” and “phase synchrony” and the distinction is necessary to avoid confusion. Onset synchrony is poor at low-frequencies and improves with increasing frequency, as shown by studies using CAP or ABR Wave 5 (e.g., Laukli et al. 1988; Sininger & Abdala 1996; Durrant and Ferraro 1999). Phase synchrony is excellent at low-frequencies and worsens with increasing frequency over 1 kHz, as shown by single-AN-fiber studies (e.g., Johnson 1980; Palmer & Russell 1986; Furman et al. 2006). Although these two frequency trends of neural synchrony may seem contradictory, they are not. Explaining why helps in understanding how synchrony affects ANOW and CAP measurements.

First, for either kind of neural synchrony to produce a voltage large enough to be recorded by a gross electrode, many fibers must fire close in time so that the unitary potentials from each fiber add constructively. The unitary potential produced by one spike from a single-AN-fiber that is picked-up by an electrode on or near the RW has two deflections, with the main negative deflection lasting about $\frac{1}{2}$ ms (Kiang et al. 1976; Prijs 1986). For the unitary potentials from many fibers to add and not have their positive deflections counteract their negative deflections, the fibers must be synchronized so that they fire within a $\frac{1}{2}$ ms period or less. This holds for both onset synchrony and phase synchrony.

ANOW depends on phase synchrony, which occurs when one or more fibers preferentially fire at the same phase on each tone cycle. Phase synchrony, as measured in single-AN-fibers, is near maximum up to about 1 kHz, then decreases and becomes negligible by ~4 kHz (e.g., Johnson 1980; Palmer & Russell 1986; Furman et al. 2006). While there is still some phase synchrony for tones up to 2-3 kHz, the $\frac{1}{2}$ ms duration of the main phase of the AN unitary potential, and the fact that the ANOW is at twice the tone frequency limits the use of ANOW to frequencies ~1 kHz and lower. Because of this, ANOWs cannot be used to find the upper phase locking limit of the human auditory system. For a 2 kHz tone, neural responses may be synchronized to be mostly within one half of the tone cycle, i.e. $\frac{1}{4}$ ms, but this response is spread out further by the $\frac{1}{2}$ ms duration of the main response lobe of the AN single-AN-fiber unitary potential. One half of a millisecond is the whole period of the 2 kHz tone and twice the period of its ANOW (which is at 4 kHz) so there is little net synchronization that can be seen in the ANOW. This limits ANOW measurement to an upper frequency of about 1 kHz, and as our results show, ANOW thresholds reflect single-AN-fiber thresholds much better at 700 Hz and below than at 1 kHz.

Onset CAPs mostly depend on the onset synchrony that occurs when many fibers fire at approximately the same time in response to the rapid onset of a stimulus. To get a measurable onset CAP, a tone onset must produce onset synchronization of many fibers within the approximately $\frac{1}{2}$ ms peak of the unitary potential, but a half cycle of a tone at a frequency below 1 kHz lasts longer than $\frac{1}{2}$ ms. If, to achieve onset synchrony, the tone onset ramp is set to be $\frac{1}{2}$ ms, then for frequencies below 1 kHz there will be significant frequency splatter and the stimulus will no longer be frequency specific. For a threshold measurement in CF regions where the threshold is not changing rapidly with frequency, the splatter of sound energy to nearby frequencies matters little because there is less energy at these nearby

frequencies than at the test frequency. However, at low frequencies where the threshold sound level increases rapidly as frequency decreases, splatter to higher frequencies may excite higher-CF neurons at lower tone levels than the tone level that excites neurons tuned to the test frequency. This is an especially acute problem in cases where there is a deep notch in the audiogram (e.g. when there are cochlear dead spots, or when opening the cochlea to make mechanical measurements in animals produces local damage). Splatter can be reduced and the stimulus made more frequency specific by turning the sound on slowly over several stimulus cycles, but for frequencies <1 kHz, this will reduce the onset synchrony and reduce the resulting onset CAP. If the onset is made slow enough, splatter will be reduced and the slow-onset tone will produce a tone-phase-locked response like that in an ANOW. With alternating-polarity tones that cancel the CM and tone rise times of two tone cycles (the rise-time used by us and many others), there is a combination of CAPs from onset frequency splatter and from ANOW-like phase locking (Eggermont & Odenthal 1974). The ANOW-like response at the onset of such a tone burst will provide some frequency specificity, but not as much as the ANOW from the ongoing response region which is not subject to splatter. As shown in Figures 6 and 7, the onset response to such a stimulus typically produces a less sensitive metric than the ANOW below 700Hz.

What is the useful frequency range of ANOW and how many dB is ANOW threshold above neural threshold?

For the 300-1000 Hz frequency range that we tested, ANOW thresholds were most reliable (i.e. were most constant relative to single-AN-fiber thresholds) at 700 Hz and below, and least reliable at 1 kHz. Since ANOW depends on neural phase synchrony to align the individual AN-fiber unitary potentials within $\frac{1}{2}$ cycle of the original tone, it was expected that ANOW would fail at high frequencies. Indeed, in our first ANOW experiment we collected data from 2 kHz tones and found such low ANOW amplitudes and correspondingly high thresholds that we discontinued using 2 kHz tones. ANOW thresholds at 1 kHz were sometimes 20 dB above single-AN-fiber thresholds, but were often much higher when the electrode was not directly on the RW (Figs. 6-7). It appears that 1 kHz is near, or above, the upper limit of the usefulness of ANOW.

At present, the lowest frequency at which ANOW thresholds might be useful is unknown. In our first ANOW experiment we also included 200 Hz tones and considered only the ANOW component at twice the tone frequency. The result showed 200 Hz ANOW thresholds to be far more than 20 dB above the single-AN-fiber thresholds, so we discontinued using 200 Hz tones. Our subsequent analysis of ANOWs (e.g. Fig. 4) shows that as tone frequency is decreased below about 400 Hz, frequency components above twice the tone frequency become increasingly prominent in the ANOW. We expect that if energy above twice the tone frequency is taken into account, then tones at 200 Hz and below will produce usable ANOW responses. Developing a method for including response components above twice the tone frequency will be an important consideration when translating ANOW to a species with a hearing range lower than cats, e.g. humans.

At 700 Hz and below, our ANOW thresholds were approximately 10-20 dB above the thresholds of the most sensitive (lowest threshold) single-AN-fibers. It is possible that with further development of the method, ANOWs will track single-AN-fiber thresholds even better. One possible direction for developing the method is to use coherence analysis (e.g. Levi et al. 1995; Dobie & Wilson 1996). It was not possible to apply this method to the present data because the coherence analysis method requires obtaining multiple sub-averages, which we did not do. We emphasize that our proof-of-concept study has focused on determining whether ANOW might be used to provide estimates of cochlear neural thresholds at low frequencies, rather than on determining the exact dB difference between ANOW thresholds and single-AN-fiber thresholds. We conclude that ANOW provides

useful information about the sensitivity of low-frequency hearing. The number of dB between ANOW thresholds and single-AN-fiber thresholds will depend on electrode placement, how many responses are averaged, the processing of the ANOW data, the criterion used to determine ANOW threshold, and other factors. These need to be worked out for each electrode location and species of interest.

One can ask: “Why are ANOW amplitudes so low?” As shown in Figure 5, the ANOW threshold at the criterion value is a few tenths of a μV while our normal criterion voltage for onset CAP thresholds is 10 μV . One reason for low ANOW amplitudes is that near threshold ANOWs are generated by fibers with CFs at the tone frequency that fire preferentially at one phase of the tone, but not at every cycle of the tone. The ongoing sound causes adaptation that lowers the ANOW response, but with onset CAPs, the fibers have not been adapted by prior portions of the tone, and a much larger fraction of the fibers fire at the onset. Furthermore, the onset frequency splatter excites fibers over a wider frequency range than the frequencies excited by an ongoing tone, which means that more AN fibers contribute to the onset CAP than to ANOW. Another reason for low ANOW amplitudes is that when two cochlear responses are averaged, the neural response in each cochlear response occurs at a different time and is divided by two in the averaging process.

Comparison of our results with previous results

Averaging responses from opposite polarity sounds to cancel the CM and yield neural responses has a long history. This technique originated soon after the advent of computer averaging of auditory responses (e.g. Peake et al. 1962). Since then, most uses of this technique have focused on the onset CAP that was revealed by cancelling CM. A cancelled-CM ongoing neural response at twice the tone frequency that appears to be an ANOW has been previously shown but not identified as a useful neural response (e.g. Eggermont & Odenthal 1974; Ferraro et al. 1994). Henry (1995) appears to be the first to have shown cancelled-CM ongoing neural responses (ANOWs) and to have done the necessary experiments to prove that these responses originated from the auditory nerve. However, Henry did not attempt to use these potentials as a metric for apical cochlear sensitivity, or anything else. The first use of quantified ongoing ANOWs as a monitor of apical cochlear health was by Dong and Cooper (2001) using responses from near the RW during experiments that measured motion in the apical turn of guinea pigs. These experiments showed that the ANOW amplitude reached a criterion value at higher levels (i.e. became worse) as the experimental preparation degraded, but they did not show the relationship of their ANOW thresholds to onset-CAP and single-AN-fiber thresholds. The present paper is the first to show ANOW thresholds in relation to onset-CAP and single-AN-fiber thresholds.

Henry (1995) used the same CM-cancellation technique to derive his “ANN residual” as we have used to derive ANOW, and our results generally agree with his. The ANOW characteristics that we found are similar to those that Henry found in his “ANN residual” in that: (1) ANOW has major energy at twice the tone frequency and little energy at the tone frequency, (2) ANOW shows adaptation, (3) ANOW shows nonlinear growth, and (4) ANOW shows “peak splitting”. Forward masking is the only response property that Henry described in the ANN residual that we have not tested for in ANOW. Henry (1995) found that for moderate-level tones the amplitude of the “ANN residual” decreased with increasing tone frequency over the range 600 Hz to 4 kHz. Our ANOW amplitude data also decreased with increasing frequency. Henry did not provide any data for tones 500 Hz and below – the range in which we found ANOW most closely paralleled single-AN-fiber thresholds.

Ferraro et al. (1994) show human data recorded from a promontory electrode and an electrode on the tympanic membrane (TM) that are almost certainly ANOWs. They averaged responses from 500 Hz, alternating-polarity tones and the resulting waveforms

show oscillations at twice the 500 Hz probe frequency as expected for an ANOW. The Ferraro et al. work was targeted at measuring summing potentials. They show ANOWs only for 500 Hz, 90 dB nHL tones, and they did not extract amplitude measures. From their Figure 2A, the amplitude of the promontory-electrode ANOW was about 3 μ V peak-to-peak which corresponds to an ANOW component at twice the tone frequency of about 1.5 μ V. This is smaller than the ANOWs we measured at high tone levels with electrodes on the cat round window (Fig. 4A). Although their ANOW was smaller, the important point is that the results of Ferraro et al. demonstrate that ANOW can be measured in humans using non-invasive tympanic membrane recordings.

Possible uses of ANOW

For frequencies of 700 Hz and below, ANOW can provide information about cochlear hearing sensitivity in a highly frequency specific manner that is difficult or impossible to obtain by any other non-behavioral method. ANOW can be applied in animals, as shown by our data. ANOW would be useful in any animal experiment when it is important to determine low-frequency hearing sensitivity and to track it over time. For example, ANOW measurements would be especially useful when opening the cochlear apex to make mechanical measurements or in using pharmaceuticals or other manipulations that can change cochlear function at low frequencies (e.g., Dong & Cooper 2001). Another use of ANOW is in animal models of hydrops that are classically defined by an enlarged scala media. Human Ménière's disease, which is associated with cochlear hydrops, often produces a low-frequency hearing loss and is expected to do the same in animals, but this has not been tested because there had been no suitable way to measure very low-frequency thresholds with cochlear gross potentials in animal models of cochlear hydrops. ANOW provides such a method.

It seems possible that ANOW will work in humans with recordings from a non-invasive electrode placed on the TM as is demonstrated by Ferraro et al.'s (1994) 500 Hz data. Before this is achieved, however, there are numerous technical problems to be overcome, with the main one being the poorer signal-to-noise ratio of ANOW recorded from human ear-canal or TM measurements. In humans, ANOW measured using an ear canal electrode (e.g., Gaddam and Ferraro 2008; Riazi and Ferraro 2008) may be useful for estimating thresholds in infants, or in conditions that make it impractical to obtain behavioral responses. ANOW measurements might also be useful in cochlear implant surgeries designed to preserve low-frequency hearing; in such cases an electrode could be put directly on the cochlea and the ANOW test used to determine if there is residual hearing at low frequencies and if this hearing is maintained during surgery. In both human and animals, the ANOW may be a useful measure to study the influence of the medial olivocochlear system on the cochlear apex.

Conclusions

We describe a novel technique to estimate low-frequency cochlear thresholds that uses ANOW responses. Our data show that at low frequencies (700 Hz and below), ANOW thresholds were mostly 10-20 dB *more* sensitive than onset-CAP thresholds and 10-20 dB *less* sensitive than the most sensitive single-AN-fiber thresholds. Considerable work is required to optimize the ANOW technique and to translate it to humans. The results found here are promising in that they show that ANOW can be used to objectively estimate thresholds at very low frequencies in a highly frequency-specific manner, a task for which other techniques work poorly, if at all.

Acknowledgments

We thank Drs. Sharon G. Kujawa and Christopher A. SHERA for comments on the manuscript. This work was supported by grants F32 DC010112, RO1 DC00235, RO1 DC03687, and P30 DC005209 from the NIDCD, National Institutes of Health.

Source of Funding: JTL and JJG are currently receiving grants from NIH.

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Figure 1.

Typical examples of cochlear responses and the auditory nerve overlapped waveform (ANOW) from an electrode on the round window. A, B: Cochlear responses from 50 dB SPL, 500 Hz tones of opposite phases. The vertical line aids visualization of the opposite response polarities in A vs. B. C: The ANOW that resulted from averaging the responses in A and B. Scale bars indicate voltage in μ V (vertical) and time in ms (horizontal). Cat 37, series 629.

Figure 2.

Example ANOWs at different sound levels. 500 Hz tone data from cat 35, series 629. Scale bars indicate voltage in μV (vertical) and time in ms (horizontal) and apply to all the waveforms on their side of the figure.

Figure 3.

Example ANOWs at different tone frequencies. Left: ANOWs. Right: Averages of the ANOW across the analysis window (horizontal bar below the 1000 Hz waveform) in blocks of 1 tone cycle, which shows two ANOW cycles. The time scale is expanded 2X. The arrow indicates a CAP offset response at 500 Hz. 50 dB SPL data from cat 32, series 389 (electrode on the round window).

Figure 4.

ANOW energy is at the second, and higher, multiples of the tone frequency. Example ANOWs transformed to the frequency domain from tones at the frequency listed above each response. Arrowheads indicate the tone frequency (open symbol), twice the tone frequency (filled symbol) which is the peak from which ANOW2f was obtained, and four times the tone frequency (grey symbol). The near absence of energy at the probe frequency shows that the CM was adequately cancelled. Cat 35, series 629, 70 dB SPL.

Figure 5.

A: ANOW and B: cochlear response (CR) magnitudes as functions of tone level. The horizontal lines in A are the ANOW threshold criteria for the frequencies indicated by each symbol. Cat 37, series 793.

Figure 6.

ANOW thresholds (thick lines) are more sensitive than low-frequency onset-CAP thresholds (thin lines) and less sensitive than single-auditory-nerve-fiber thresholds (gray circles). The standard onset-CAP thresholds at 2 kHz and above used a different criterion than the onset-CAP thresholds at 1 kHz and below (see methods). Data from an electrode on the round window (RW) = solid lines. Data from an electrode not on the RW = dashed lines. The cat number is in the lower left of each panel.

Figure 7.

The amount by which ANOW threshold was lower than the onset CAP threshold. Symbol indicates the electrode position. RW = round window.