ELECTROCOCHLEOGRAPHY MEASURES FROM THE EAR CANAL OF AWAKE CHINCHILLAS

by

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To my family and friends who have supported me through everything

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LIST OF ABBREVIATIONS

SNHL	Sensorineural hearing loss
ECochG	Electrocochleography
CAP	Compound action potential
CM	Cochlear microphonic
SP	Summating potential
IHC	Inner hair cells
OHC	Outer hair cells
NH	Normal hearing
CA	Carboplatin
GE	Gentamicin

ABSTRACT

Disabling hearing loss is a problem around the world, with the World Health Organization estimating that 466 million people worldwide have disabling loss, and that this number is expected to increase to over 900 million people by 2050. There are different types of hearing loss, but sensorineural hearing loss (SNHL) is the most common and results from damage to the inner ear. The audiogram is the most common test used to diagnose hearing loss, but it is limited in that it can only identify a shift in hearing sensitivity (thresholds), i.e., it cannot identify the cochlear location causing SNHL. The electrocochleogram (ECochG) is an evoked response consisting of several summed responses of electrical potentials from within the inner ear. Several components represent activity from different places in the inner ear: the compound action potential (CAP) is the summed onset response of auditory nerve fibers, the cochlear microphonic (CM) is the AC response of the hair cells (primarily outer hair cells), and the summating potential (SP) is the DC hair cell response (primarily inner hair cells). Most ECochG responses in humans are collected non-invasively (e.g., from the ear canal or ear drum), whereas most ECochG responses in animal models are collected invasively (e.g., from the cochlear round window).

In this project, we aimed to bridge this gap by recording non-invasive ECochG responses from awake chinchillas. We first started by calculating standard ECochG metrics from existing data across different forms of SNHL. Next, we tested the feasibility of recording non-invasive ECochG responses from the ear canals of awake chinchillas. Finally, we defined and calculated additional metrics from ECochG responses to further help in identifying location(s) of SNHL. The ability demonstrated here to record non-invasive ECochG responses from awake animals increases the translational applicability of pre-clinical SNHL animal models by permitting detailed cochlear assessments at multiple time points post exposure. Detailed ECochG measures can advance hearing science and audiology by helping to identify the location of damage causing the hearing loss, which can ultimately allow for more individualized treatment.

1. INTRODUCTION

1.1 Overview of the Peripheral Auditory System

There are three parts that make up the ear: the outer ear, the middle ear, and the inner ear. The outer ear consists of the pinna and the ear canal. The middle ear includes the ear drum and three bones called the ossicles that connect to the ear drum and transfer sound energy to the inner ear. The inner ear consists of the cochlea and the semicircular canals, with the former part of the auditory system and the latter part of the balance system. Within the cochlea are two different types of hair cells, inner hair cells (IHC) and outer hair cells (OHC). The inner hair cells help convert sound to an electrical signal that is passed to the brain via the auditory nerve, whereas the outer hair cells are unique electromotile cells that amplify soft sounds to provide excellent sensitivity and frequency resolution. There are specific structures in the brain that help in processing sound, but most forms of hearing loss stem from issues in the peripheral auditory system which includes everything up to the auditory nerve.

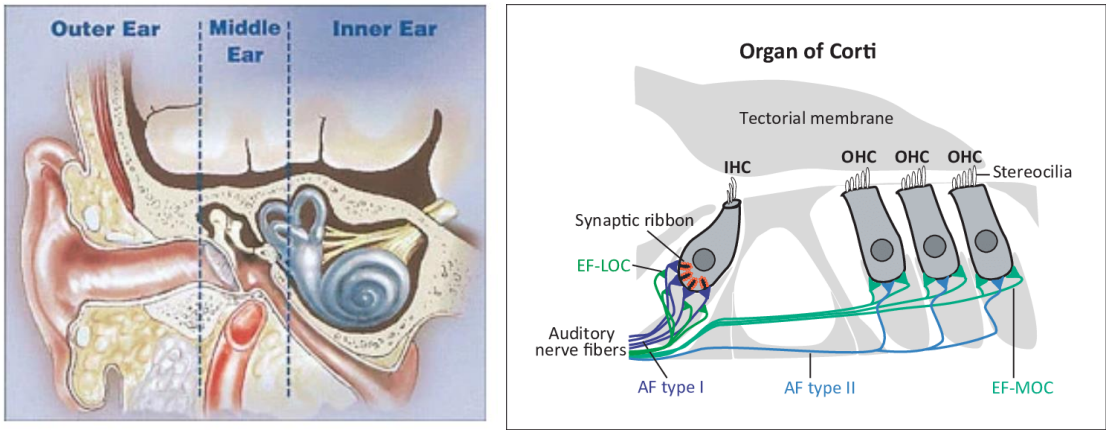

Figure 1.1 The left figure shows a diagram of the ear with the different parts: outer, middle, and inner ear [1]. The right figure shows a diagram of the organ of corti in the inner ear which has the inner and outer hair cells [2].

1.2 Importance of Hearing Loss Location

There are several different types of hearing loss, including congenital loss, conductive loss, sensorineural loss, age-related loss, and mixed loss. According to the World Health Organization, about 466 million people worldwide have disabling hearing loss, and it is estimated to increase to

over 900 million people by 2050 [3]. Sensorineural hearing loss (SNHL) is the most common type of hearing loss and results from damage to the inner ear [4]. There are many different potential causes for SNHL, including but not limited to excessive noise exposure, infections, trauma, and ototoxic drugs [4].

There are many different tests that measure hearing, but one of the most common tests is the audiogram. The audiogram tests pure-tone hearing thresholds, or the softest a sound needs to be in order to be heard, at different frequencies [5]. Based on the results of the audiogram, it is possible to identify the degree of hearing loss, and sometimes the type of hearing loss [5]. However, the audiogram is a limited tool and alone cannot be used to identify the specific location of hearing loss. It is important to identify the location of the hearing loss as damage to different parts or structures changes how hearing is affected [6] and changes how best to treat or account for the hearing loss. For instance, damage to the OHCs may be remedied in part by increasing the sound level going into the ear via a hearing aid whereas damage to the auditory nerve means the signal is not correctly being passed to the brain no matter how loud it is.

1.3 Electrocochleography

The electrocochleogram (ECochG) is a measure of the summed responses of electrical potentials in the inner ear evoked by different stimuli. There are three components that make up the electrocochleogram: the compound action potential (CAP), the cochlear microphonic (CM), and the summating potential (SP). The CAP is the summed response of auditory nerve fibers [7]. A click stimulus activates the entire length of the cochlea and can test the overall response of the cochlea whereas a tone stimulus activates specific portions of the cochlea and can test the response of specific sections (at least for soft and moderate sound levels for which spread of excitation is limited). The CAP response is usually a large negative peak near the onset of the stimulus followed by a positive peak, and the difference in the peak amplitudes is the response amplitude. The CM is the alternating current (AC) response of the hair cells in the cochlea, primarily the OHCs, and the oscillations of the response follow the frequency of the stimulus [8]. The SP is a direct current (DC) potential that can be identified as a baseline shift in the CM and is also the response of the hair cells with more contribution from the IHCs [8].

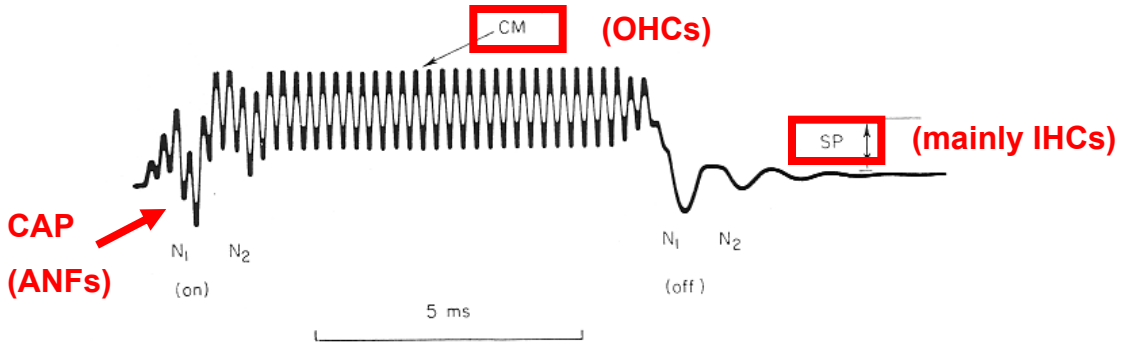

Figure 1.2 Typical ECochG response to a tone burst showing the three different components: compound action potential (CAP), cochlear microphonic (CM), and summating potential (SP) [9]. The CAP is the summed response of auditory nerve fibers (ANFs), the CM is primarily the response from the outer hair cells (OHCs), and the SP is primarily the response of the inner hair cells (IHCs). It should be noted that the CAP and SP are often more easily measured using the average of alternating polarity responses.

1.3.1 In Humans

Electrocochleography in humans is used to diagnose, assess, and monitor inner ear disease, measure and monitor auditory nerve function during surgery, and to help diagnose, assess, and monitor Meniere's disease [10]. ECochG measures are sometimes used during cochlear implantation surgeries, and because an invasive surgery is already being performed, an electrode can be placed at the round window to record responses [11]. However, most other ECochG measurements are performed non-invasively, most often using one of two types of electrodes: a gold-foil tiptrode or a tymptrode [10]. The tiptrode sits in the ear canal while the tymptrode is placed on the surface of the tympanic membrane (i.e., ear drum). The tymptrode usually produces a larger signal compared to the tiptrode since it sits closer to the source, but it is harder to place and not as comfortable for the patient compared to the tiptrode. For this reason, the present work only explores the use of tiptrodes.

1.3.2 In Animals

Animal models of different types of hearing loss are often used when studying hearing loss in part because more invasive procedures can be performed on animal models that cannot be performed on humans [12]. Another reason is that animal models can target and damage specific structures, such as only damaging the IHCs but not the OHCs, in order to see how those structures affect the overall response and what role they play in different hearing loss etiologies. There are

many different tests for measuring different aspects of hearing, but the ECochG is still one of the most used physiological tests in animals to assess damage and function of the inner ear. Within the overall ECochG response, the CAP is most often studied in models of SNHL, and the response is recorded invasively via an electrode placed at the round window [13], [14]. Because the invasive electrode is placed right near the source of the response, the signal is clear and well defined whereas non-invasive recording electrodes have more noise and smaller signals to begin with. For this reason, there are few animal studies that try to record non-invasively when invasive recordings are possible and provide better responses. However, there are limitations to only using invasive measures in animals, one of which is that invasive measures requires sacrificing the animal which removes the pre-post within animal controls.

1.4 Animal Model

The chinchilla, or more specifically *Chinchilla lanigera*, has been used as an animal model for hearing loss for over 50 years [12]. There are many benefits to using the chinchilla as a model for hearing loss which include the fact that chinchilla's hearing frequency and intensity sensitivity are more similar to humans than other rodent models, they have the ability to be trained behaviorally with acoustic stimuli that are relevant to human hearing, they have a docile nature that allows many physiological measures to be made in an awake state, and there is the ability to model various types of conductive and sensorineural hearing losses which mimic pathologies observed in humans [12]. For these reasons, as well as the fact that the chinchilla model of hearing was well established in the lab, the chinchilla model was used to study measures of ECochG.

1.5 Addressing the Gap in Literature

As mentioned above, most human ECochG responses are recorded non-invasively and awake whereas most animal responses are recorded invasively and anesthetized. There have been a few studies that used non-invasive methods to collect ECochG responses in different animal models. Quddusi and Blakely studied ECochG responses in anesthetized mice and compared a more invasive needle electrode placed through the tympanic membrane that rested on the promontory of the cochlea and a less invasive electrode that consisted of wet cotton placed on the end of a wire with the cotton then being placed on the tympanic membrane [15]. Durant and Ronis

studied ECochG responses in anesthetized guinea pigs and similarly compared responses from an invasive electrode placed in the cochlea and a non-invasive electrode of wet cotton that was placed on the ear drum [16]. Finally, Campbell et al. studied four different types of non-invasive electrodes, two of which included the gold-foil tiptrode and a tymptrode, and compared them to an invasive needle electrode in anesthetized chinchillas [17]. All three of the studies found that measurement using the non-invasive electrodes were possible, but that they were far more variable than the invasive electrodes. For example, the Campbell study reported that tymptrodes provided reasonably reliable measures but often led to punctured ear drums, whereas the tiptrodes often did not provide any reliable measures (e.g., they only showed limited summary data, and did not show any waveforms to confirm the accuracy of these cochlear recordings).

Because there are so few studies that use non-invasive methods for recording ECochG responses in animals, and the few that do studied anesthetized responses, it is difficult to compare results from animal studies to human studies and applications, aside even from specific species differences. Furthermore, there are other benefits to using non-invasive methods, such as not having to sacrifice an animal to obtain the measurements which allows for pre-post measures in animals. Another benefit of non-invasive methods is that they can be done on awake animals which allows for a greater number of time points to be measured compared to anesthetized procedures (e.g., to study day-by-day trends that might permit mechanistic insight). For these reasons, this study aimed to record non-invasive ECochG responses from awake chinchillas.

1.6 Research Questions

Note: The original goal of the thesis project was to compare and contrast ECochG measures (ear drum and ear canal) in anesthetized and awake chinchillas, and to establish the validity of the best measure. However, the COVID-19 pandemic and associated 4 month lab closure and related restrictions forced the project scope to be adapted to include ECochG analyses of existing data, and only an initial feasibility demonstration of tiptrode ECochG measures in the ear canals of chinchillas.

The first Aim of this study (Chapter 2) addresses whether metrics from ECochG responses are sensitive and accurate enough to identify not only a hearing loss, but to identify the location of the loss as well. This involved the re-analysis of a previously collected (but not analyzed in this detail) data set from a former PhD dissertation in the lab [18]. This re-analysis of previous data was

pursued in the spring and summer of 2020 when the lab was closed due to the COVID-19 pandemic. The second Aim (Chapter 3) addressed here is whether it is feasible to collect non-invasive ECochG responses from the ear canals of awake chinchillas that are reliable and clearly cochlear. A final Aim (Chapter 4) addresses whether more advanced ECochG metrics agree with, and/or provide more information, about hearing loss compared to the more standard metrics.

2. ELECTROCOCHLEOGRAPHY METRICS TO IDENTIFY HEARING LOSS

2.1 Existing Data Source

The ECochG data analyzed in this Aim was previously collected in our lab from the round window of anesthetized adult male chinchillas during auditory-nerve experiments using animals with either normal hearing or ototoxic-induced SNHL [18]. These data were collected for the purpose of screening for hearing loss based on the CAP, and as such certain recording settings (e.g., 300-3000 Hz bandpass filter) were not ideal for some of the broader ECochG measures (e.g., SP), as discussed below. As such, the results for the SPss metric based on these data should be interpreted with caution – they are merely presented here to demonstrate the computations that have been designed to compute SP from future data sets with a lower high-pass cutoff that will allow better measurements of the SPss metric. Note that an alternative SP metric is explored in Chapter 4, based on the onset response from clicks.

2.1.1 Stimuli Used

There were two stimuli used to elicit the ECochG responses: clicks and tone bursts. The clicks were 0.04 ms in duration and were presented in alternating polarity. Because the responses were recorded from a low-impedance silver electrode placed at the round window, only 20 sets of each polarity were presented to obtain a clear response [18]. The tone bursts were 10 ms in duration (1 ms rise/fall times), and were presented at either 0.5, 1, 2, 4, or 8 kHz. Like the clicks, the tones were presented in 20 sets of alternating polarity. Both clicks and tones were presented once every 50 ms (i.e., 20 stimuli/sec). A range of sound levels were used for both clicks and tones (starting from below threshold up to suprathreshold levels), which included 13 attenuations in 5-dB steps.

2.1.2 Chinchilla Groups

There were three different hearing types: normal hearing (NH), carboplatin induced hearing loss (CA), and gentamicin induced hearing loss (GE). Carboplatin is a chemotherapy drug with known ototoxic effects specific to inner hair cells (IHCs) in chinchillas [19], [20]. Gentamicin is an antibiotic with known ototoxic effects for both inner and outer hair cells with a preferential

effect on outer hair cells (OHCs), and the low dose given was shown to only affect the OHCs [21], [22]. There were 3 chinchillas with NH, 3 chinchillas with CA, and 4 chinchillas with GE whose electrocochleography (ECochG) responses were analyzed.

2.2 Defining Metrics

2.2.1 Compound Action Potential

The compound action potential (CAP) is the combined neural response from auditory nerve fibers (ANF) that fire synchronously. The response is found near the onset of a stimulus as this is when most fibers fire together in response to the stimulus. This metric is often used to measure "cochlear status". The response is often identifiable as a significant negative peak near the onset of the stimulus followed by a positive peak, and the amplitude difference between the two peaks (i.e., peak to peak) is the amplitude of the CAP.

2.2.2 Summating Potential

The summating potential (SP) is the combined DC shift in the overall ECochG response. This shift is due to the activity of the IHCs, and as such this metric is used as a way to estimate the health of the IHCs. The response is identified as the average shift from baseline in the (pre-response) steady state portion of the ECochG response.

2.2.3 Cochlear Microphonic

The cochlear microphonic (CM) is the combined AC response in the overall ECochG response due to the activity of the OHCs. The frequency of the response usually follows the acoustic stimulus fairly well, which is why it is compared to a microphone. The response is best found in the steady state portion of the ECochG response, and the amplitude is the difference between the maximum point and minimum point of the AC response.

2.3 Calculating Metrics on Individual Data Files

For each chinchilla there were varying numbers of individual data files that were collected at different time points throughout the anesthetized procedure in response to either clicks or tones

at different levels of sound attenuation (dB SPL was computed for tones as the difference between the calibrated SPL at the tone frequency minus the attenuation; for clicks, dB SPL was computed as the difference between the average calibrated SPL across frequencies up to 10 kHz and the attenuation level). Each of these data files was loaded and the responses to the stimulus were analyzed.

In order to calculate the measures of interest, three separate windows were defined, as shown by the vertical yellow lines in Fig. 2.1 below, within the overall time of the recorded response which was 25 ms. The first was a baseline window which was defined from 0 ms up to the stimulus onset, which was at about 7.5 ms for both clicks and tones in these data. The next window was the onset window which was defined as the end of the baseline window plus 5 ms. The final window was the steady state window which was defined as the end of the onset window plus 5 ms. The windows were defined as such because an initial onset response to the stimulus, whether click or tone, should occur within the first 5 ms after onset, and the tones were 10 ms long, thus the steady state window was an additional 5 ms.

Once the windows were defined, the measures could be calculated. The first step was to separate out the positive and negative polarity responses, average them respectively to have the polarity average responses, and then take the mean of the positive and negative responses together to create a grand average response. The grand average response was then corrected for any offset from zero in order to make plotting the response easier. The offset was determined as the mean of the response in the baseline window, and this was subtracted from the total average response to correct for the offset.

To calculate the amplitude of the CAP, N1 was determined to be the minimum value in the onset window. Once N1 was found, P1 was determined as the next positive peak in the onset window. The difference between P1 and N1 was the amplitude of the CAP (in millivolts). If there was no positive peak after N1, then the maximum in the onset window was determined and the difference between the maximum and N1 was set as the peak-to-peak amplitude. The CAP was calculated for both clicks and tones at all sound levels.

Because clicks are very short in duration, the ECochG response to clicks is mainly an onset response with little to no steady state response, and thus the standard SP and CM were not calculated for click responses (see Chapter 4 for advanced measures of SP and CM from click responses that were explored later). The standard SP for the tone responses was calculated as the

mean of the average response in the steady state window. (Note that for this data set, the SP measure is less meaningful because the recordings were made using a 300-3000 Hz bandpass filter that eliminates most of the SP). The CM was calculated from the two polarity average responses individually. The difference between the maximum and minimum points was found for both the positive and negative polarities, and the average difference of the two polarities was taken as the CM.

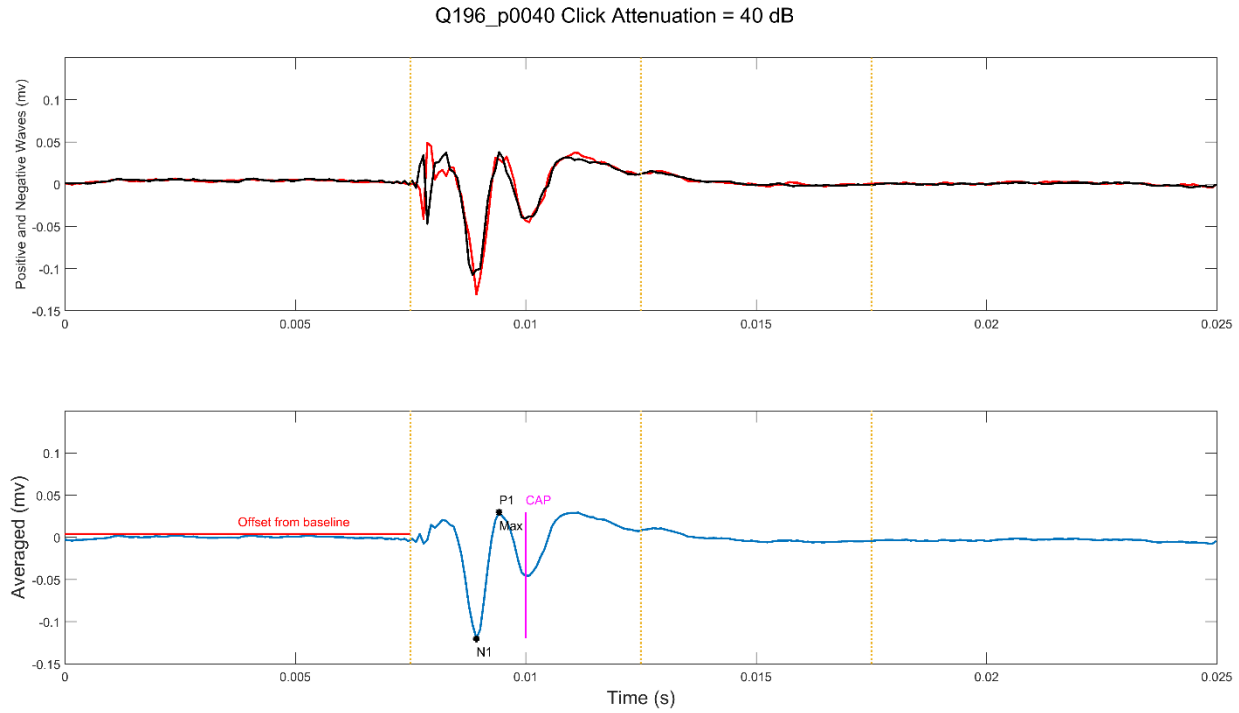

Figure 2.1 Example of a normal hearing click response showing the metric calculations. The vertical dotted yellow lines delineate the different windows. The top subplot shows the positive and negative polarity averages in mv, and the bottom subplot shows the averaged response in mv.

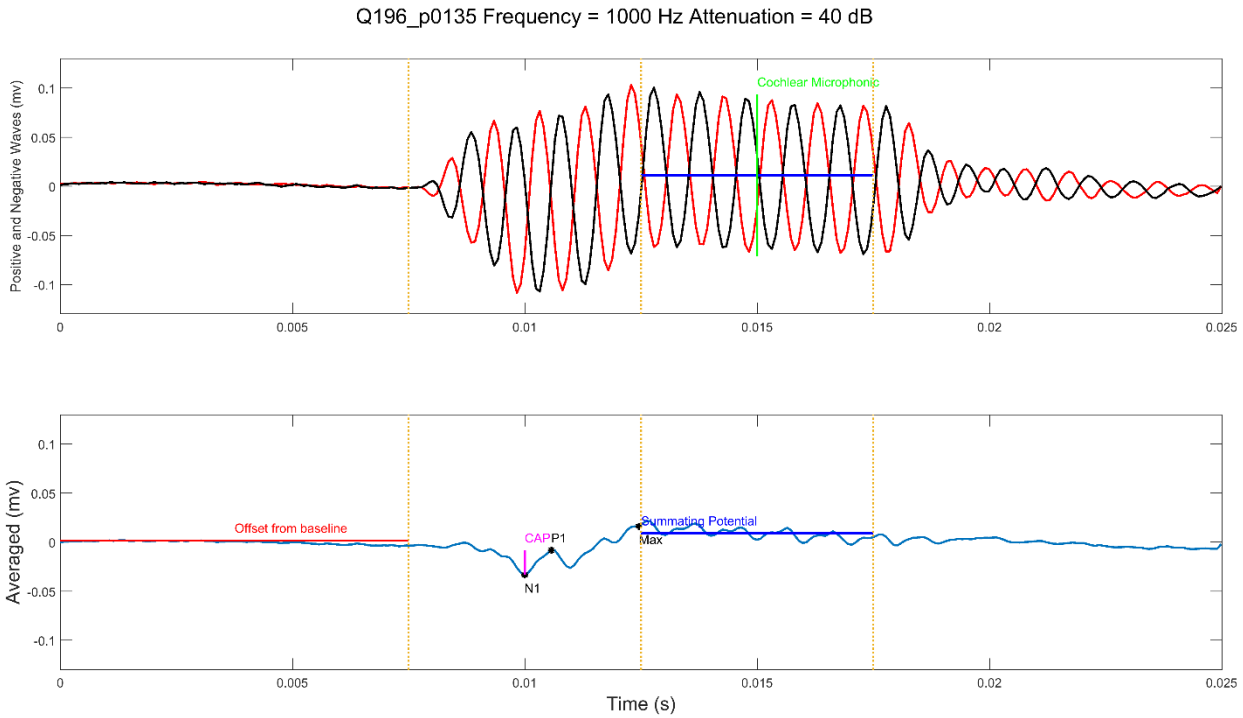

Figure 2.2 Example of a normal hearing tone burst response to 1 kHz frequency. The vertical dotted yellow lines delineate the different windows. The top subplot shows the positive and negative polarity averages in mv from which the CM is calculated in the steady state window. The bottom subplot shows the averaged response in mv from which the CAP and SP are calculated.

2.4 Calculating Thresholds

After the metrics were calculated for all files for all chinchillas, thresholds for the different metrics were determined. To find the threshold of the CAP amplitude, the peak-to-peak amplitude of the baseline of the averaged response was first calculated for all sound levels. The mean and standard deviation of the baseline amplitudes across SPLs were calculated, and the threshold criterion was set as the mean plus two standard deviations (i.e., the noise floor). For the SP threshold, the noise floor was determined from the mean of the baseline window of the averaged response, and calculated for all sound levels. The mean and standard deviation of the baseline averages were calculated, and the threshold was set as the SPL for which the SP rose above the mean plus two standard deviations. To determine the threshold for the CM, the amplitudes of the baseline for the positive average and the negative average were calculated. The amplitudes for the two polarities were combined and then the mean and standard deviation were calculated with the threshold set as the SPL for which the CM rose above the mean plus two standard deviations.

2.4.1 Measures Versus Level

Once all of the thresholds for the different metrics were determined, the metrics were plotted versus sound level (in dB SPL) with the threshold criterion in order to determine at what (interpolated) sound level the metric crosses threshold. For CAP and CM, if there were multiple points where the metrics crossed threshold, the first and lowest sound level that crossed was recorded. For the SP, if there were multiple points, the last and loudest sound level was recorded due to the fact that when the data was collected, it was not optimized to capture the SP. For all three metrics, if there were no point where the metric crossed threshold, it was recorded as not a number (NaN). All three metrics were plotted versus sound level on the same figure, with the SP and CM metrics plots blank for click responses, as shown in Figs. 2.3 and 2.4 below.

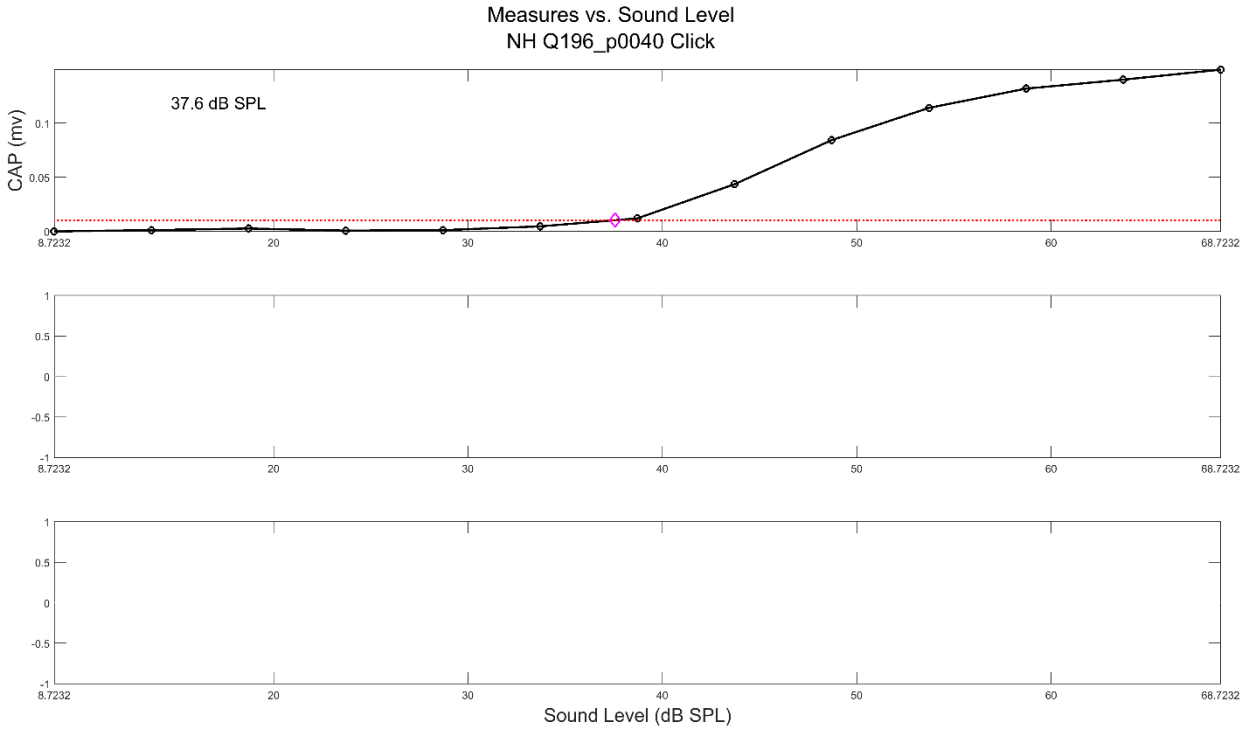

Figure 2.3 Example of a measures versus sound level plot for a NH click response. Only the CAP metric is plotted since it's a click response. The horizontal red dotted line is the threshold and the pink diamond where the two lines intersect is the sound level at which it crosses threshold, and this level is plotted in the upper left corner.

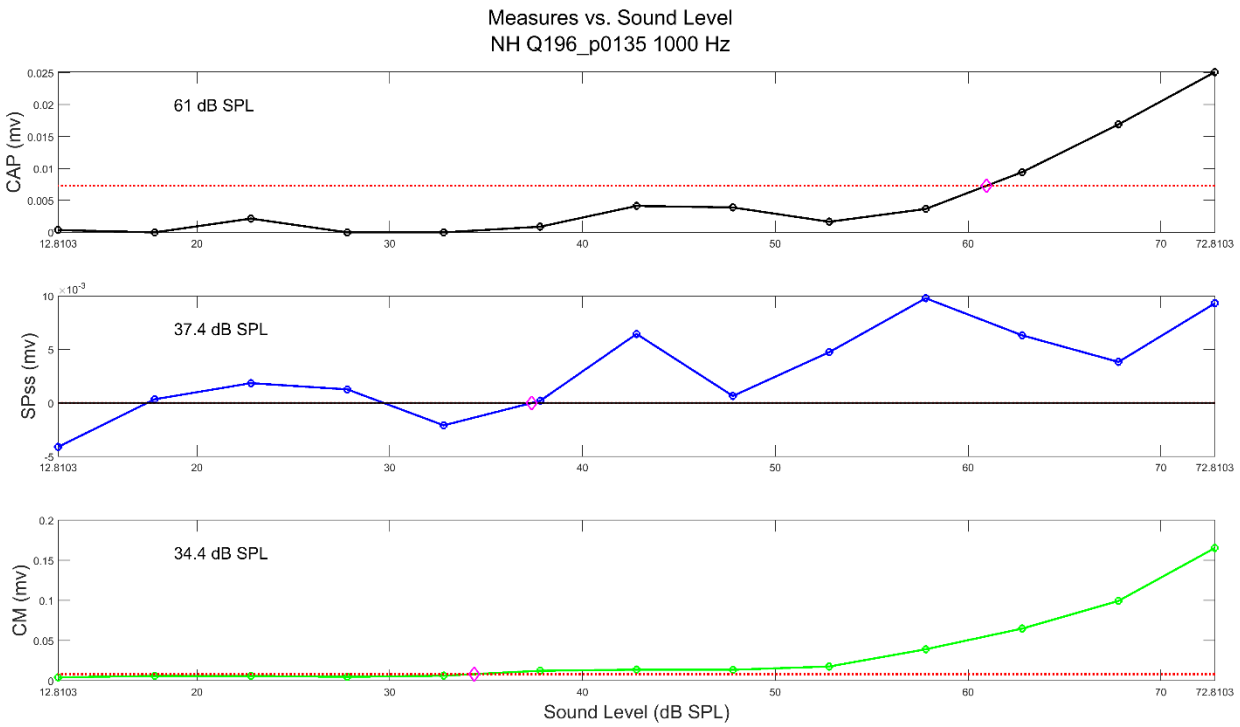

Figure 2.4 Example of a measures versus level plot for a NH 1 kHz tone burst response including the CAP, CM, and SPss which is the steady state SP response. The horizontal red dotted lines are the thresholds for the respective metrics. The pink diamonds mark where the metrics cross threshold, and the sound levels are plotted in the upper left corners of each subplot.

2.4.2 Audiograms

An audiogram is a graph that plots the lowest sound level needed to produce a response just above a given threshold criterion across different frequencies. Since a range of frequencies were tested, audiograms for the different metrics could be plotted. The sound levels where the metrics crossed their respective threshold criteria calculated in the previous section were plotted for the five different frequencies tested, as shown in Figs. 2.5-2.7 below. If the threshold was NaN for any frequencies, it was marked as a red "X" on the x-axis for the given frequency.

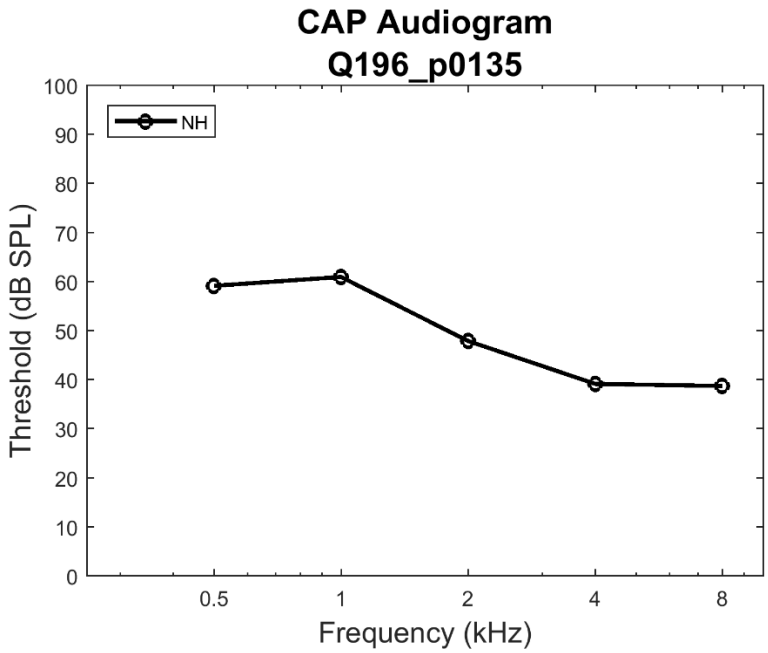

Figure 2.5 An audiogram for the CAP metric from a NH chinchilla. The sound levels where the metric crossed threshold in the previous measures vs. level plots for the different tone burst frequencies are plotted versus frequency. The circle denotes that it is a NH chinchilla.

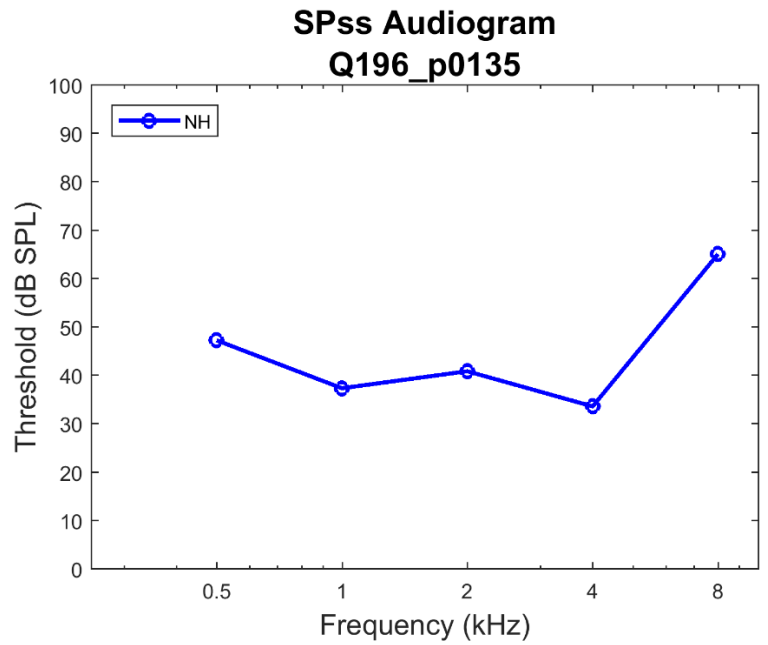

Figure 2.6 An audiogram for the SPss metric from a NH chinchilla. The sound levels where the metric crossed threshold are plotted versus frequency.

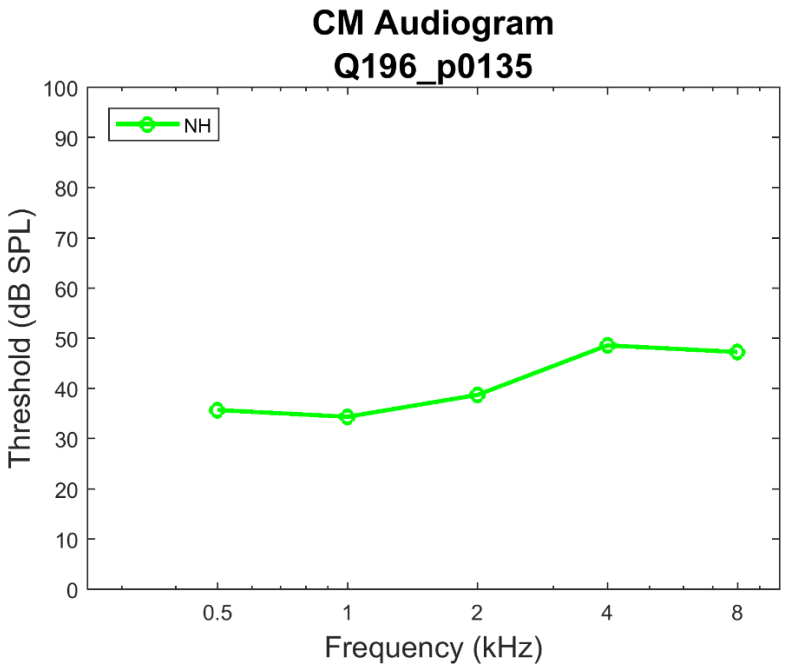

Figure 2.7 An audiogram for the CM metric from a NH chinchilla. The sound levels where the metric crossed threshold are plotted versus frequency.

2.5 Averaging Metrics across Animal and Hearing Type

After all of the metrics, thresholds, and corresponding figures were calculated and plotted for all of the individual data files for all of the chinchillas, the metrics were then averaged across files within the same chinchilla, and then the averages across chinchillas with the same hearing type were calculated. Though the metrics were collected within the same overall range of attenuation levels, the actual sound level in dB SPL differed depending on the maximum calibration level. Because of the differing sound levels, in order to average the metrics versus level, the sound levels first had to be sorted into bins. Since the attenuation levels were always in 5 dB steps, the possible sound levels ranged from -10 to 120 dB SPL in 5 dB steps. The sound levels for a given file were rounded to the nearest 5 dB step, meaning that there was at most a 2.5 dB difference between the actual sound level and the sound level used for the averaged metrics versus level. Once the metrics were sorted into sound level for all the files for a given chinchilla, they were averaged respectively to create three average metrics for a chinchilla that were then plotted versus sound level, as shown in Figs. 2.8 and 2.9 below. If data were only available from a single animal at a given SPL (e.g., lowest or highest SPL), then the average was not considered and NaN was used. After all of the individual chinchilla metrics had been averaged, the metrics for

chinchillas with the same hearing type (NH, CA, and GE) were averaged and plotted versus sound level, shown in Figs. 2.10 and 2.11 below. The averages for the three hearing types were then plotted versus sound level in order to compare trends across hearing type which is shown in Figs. 2.12 and 2.13 below.

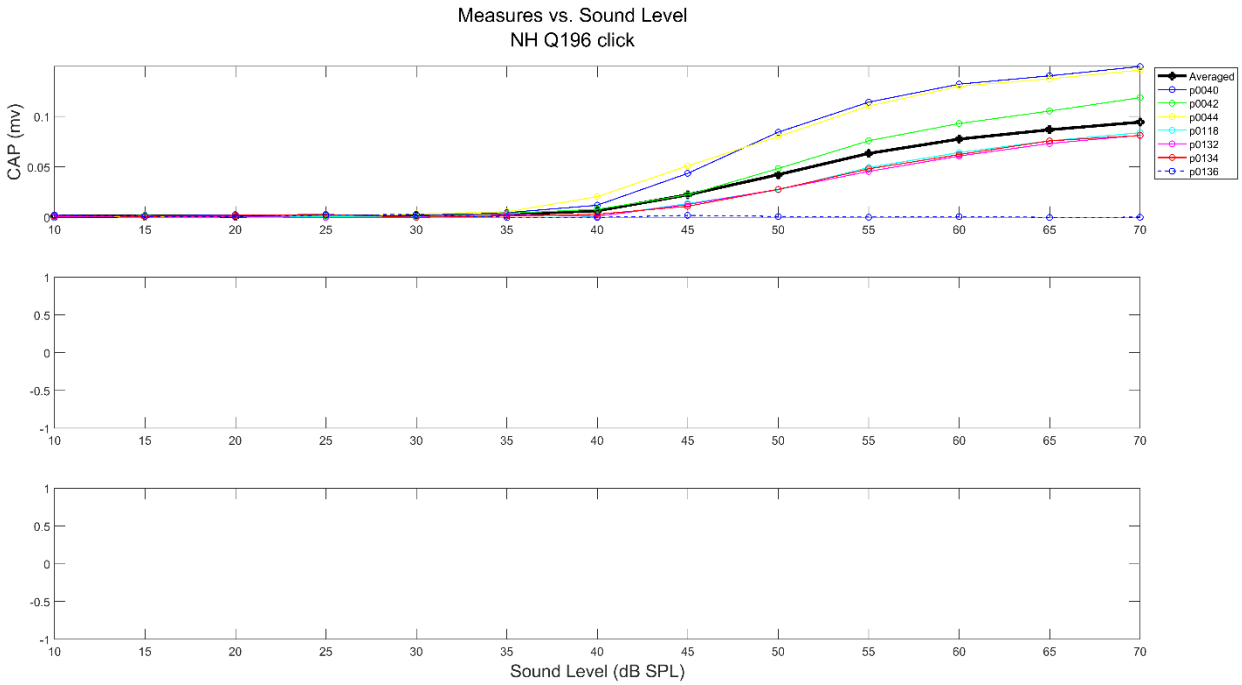

Figure 2.8 Example of an average measure versus level plot for an individual NH chinchilla for click responses. The colored lines represent the different measures vs. level lines for individual data files and the thicker black line represents the average of these individual files. The legend to the right of the plot indicates which data files correspond to which lines on the plot.

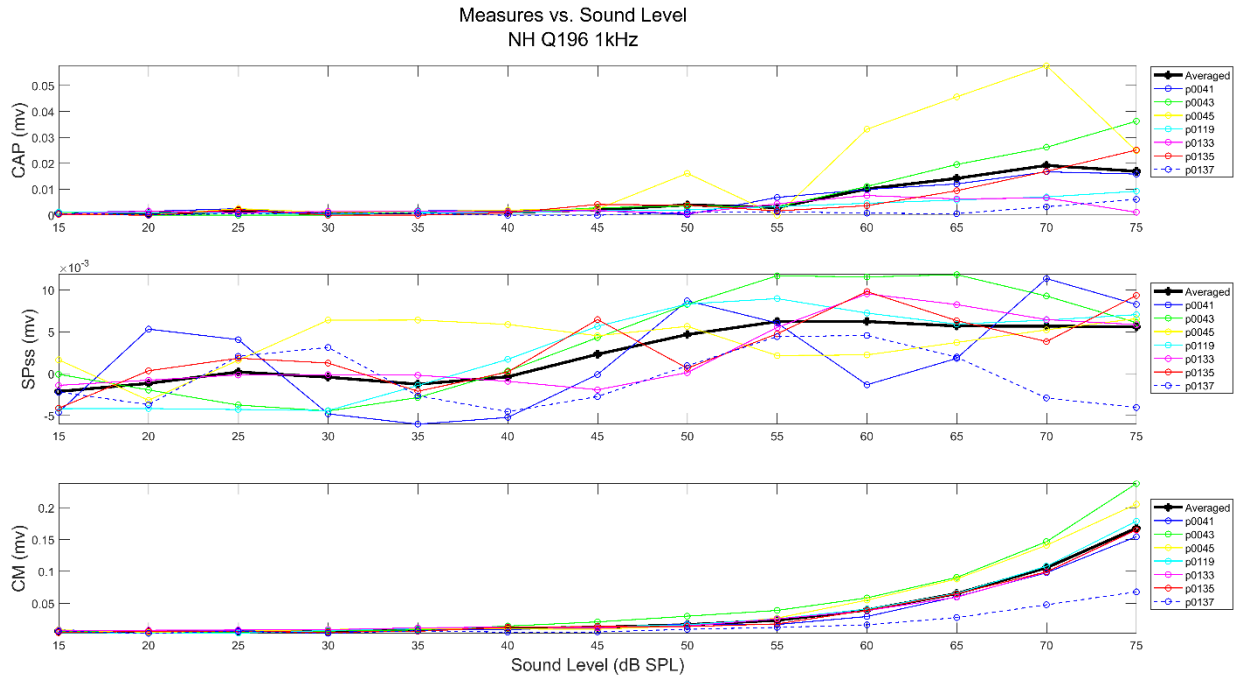

Figure 2.9 Example of an average measures versus level plot for an individual NH chinchilla for 1 kHz tone responses. The colored lines represent the individual data file measures and the thicker black lines represent the average of these files for the three different metrics. The legend to the right indicates which files correspond to which lines on the plot.

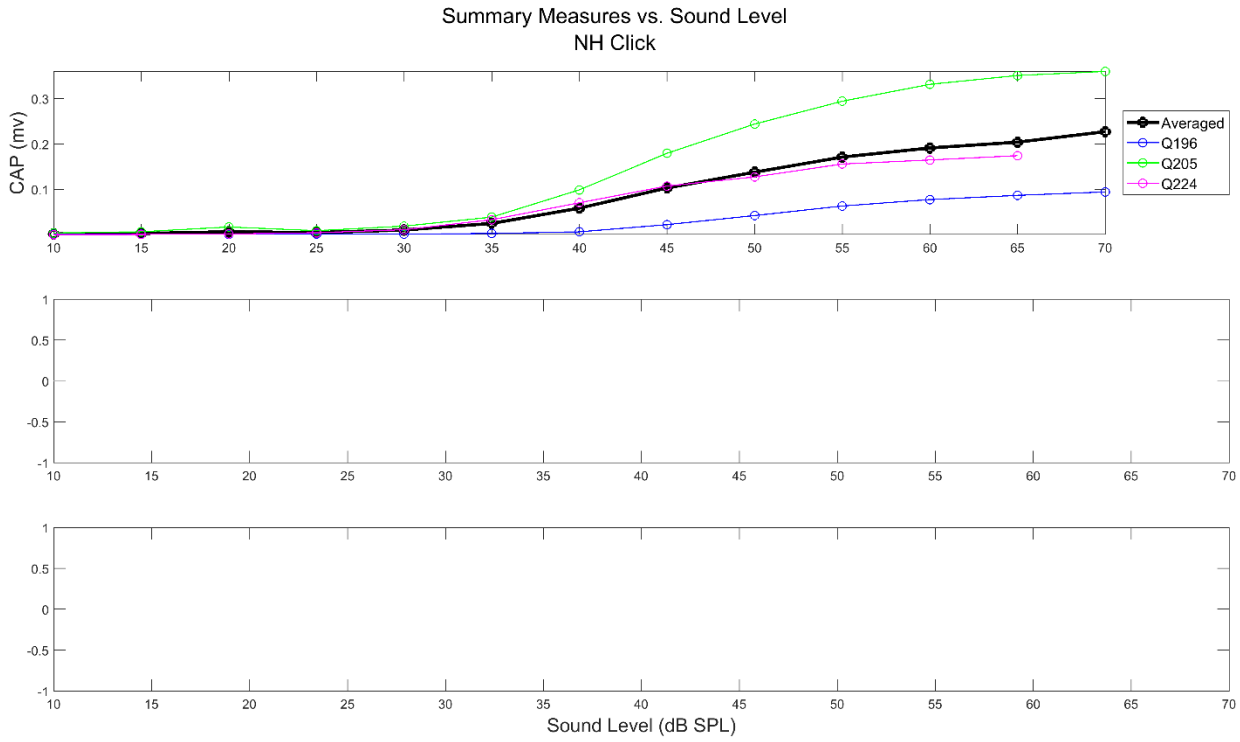

Figure 2.10 Example of an average measure versus level plot for click responses across chinchillas with the same hearing type (NH). The colored lines represent the individual chinchilla averages and the thicker black line represents the average of these chinchillas.

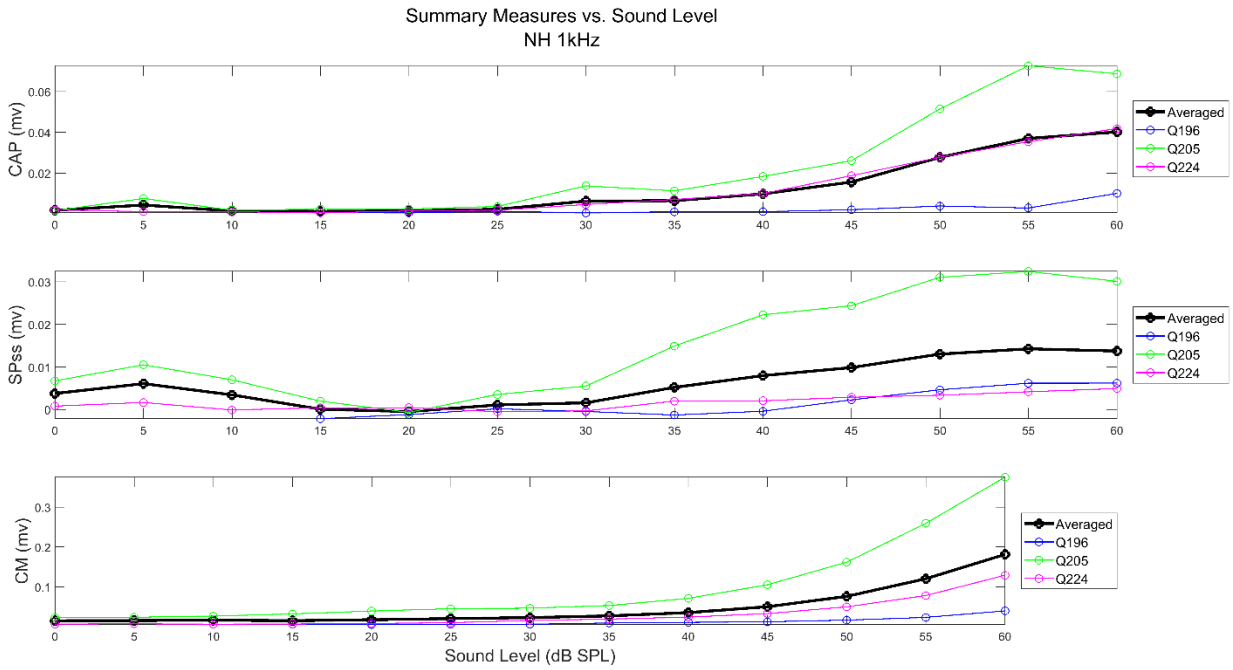

Figure 2.11 Example of an average measures versus level plot for 1 kHz tone responses across chinchillas with the same hearing type (NH). The colored lines represent the individual chinchilla averages and the thicker black lines represent the average of these chinchillas.

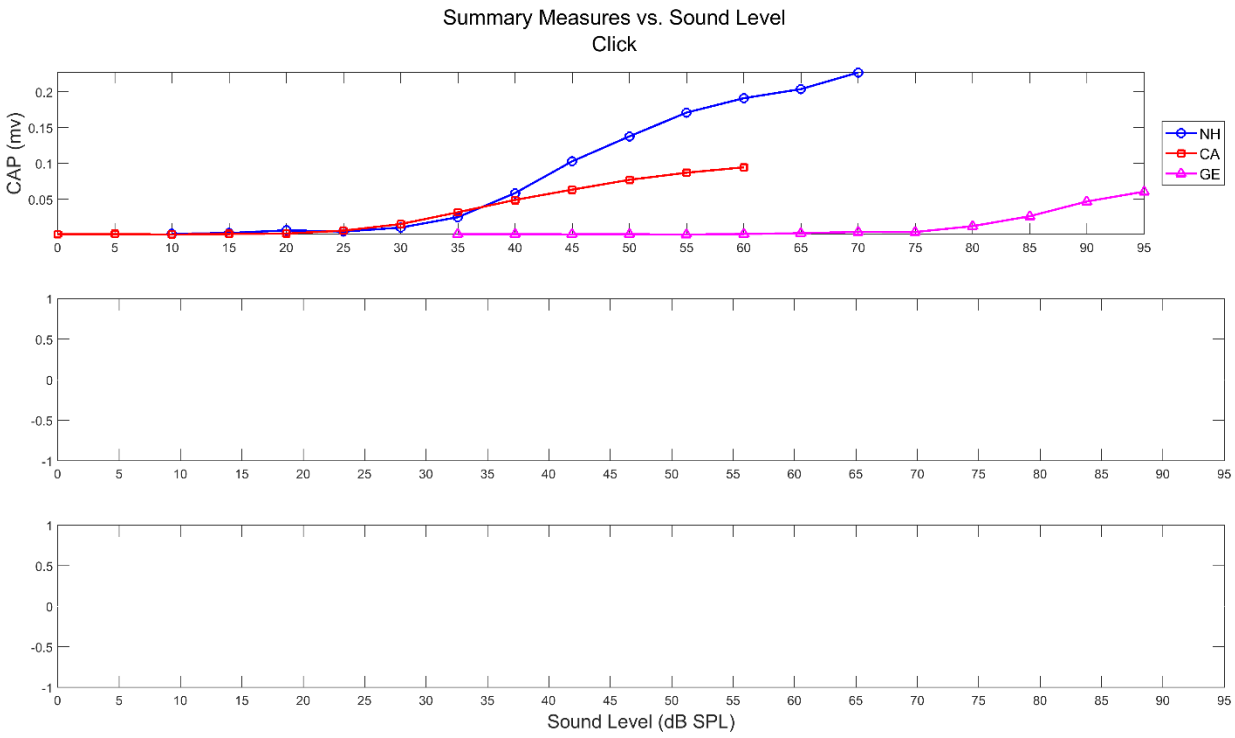

Figure 2.12 Plot comparing the average measures versus level responses for the three different hearing types to a click stimulus. The different line colors and symbols delineate the different hearing types, with the legend to the right of the plot.

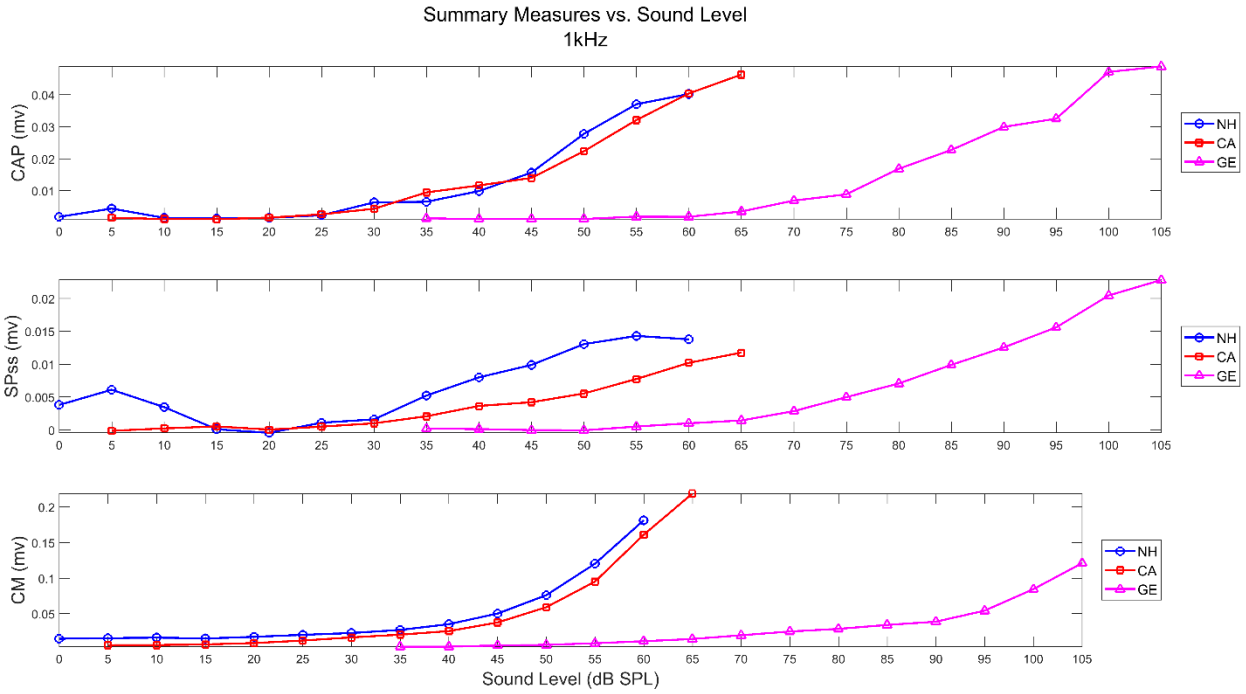

Figure 2.13 Plot comparing the average measure versus level responses of the different hearing types to a 1 kHz tone burst. The different line colors and symbols delineate the different hearing types, with legends to the right of the plots.

For the audiograms, because the same stimuli of clicks and range of tone frequencies were used, the values for the individual audiograms were averaged across the same stimulus. All of the values for a given chinchilla were averaged to create three averaged audiograms for the three metrics respectively, an example of which is shown in Fig. 2.14 below. Once the individual chinchilla averages were calculated, the values were averaged for chinchillas with the same hearing type to create audiograms of the three metrics for a given hearing type, as shown in Fig. 2.15 below. Finally, the averages of the three hearing types were plotted together to compare thresholds across type, shown in Figs. 2.16-2.18 below.

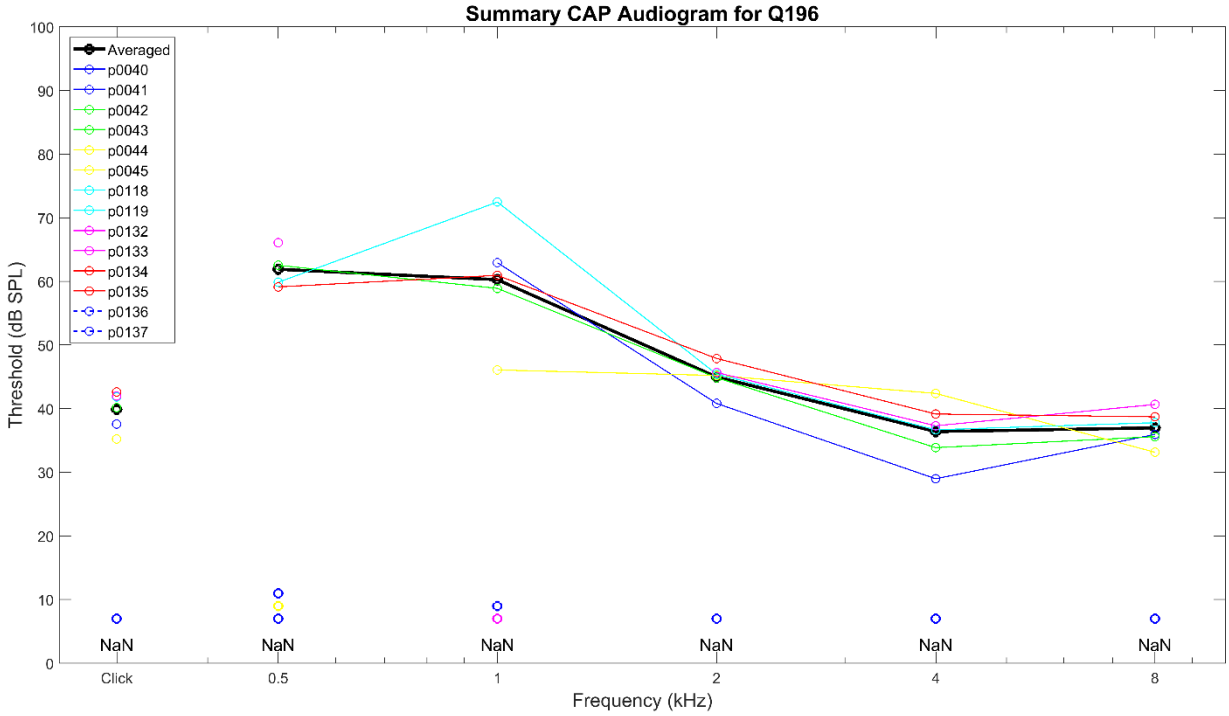

Figure 2.14 Example of an average audiogram for one metric across one individual chinchilla for click and tone responses. The different line colors indicate which lines correspond to which individual data files. The thicker black line represents the average of the individual audiograms. If any individual files did not cross threshold for a given stimulus, the corresponding line color was plotted above the NaN along the bottom axis.

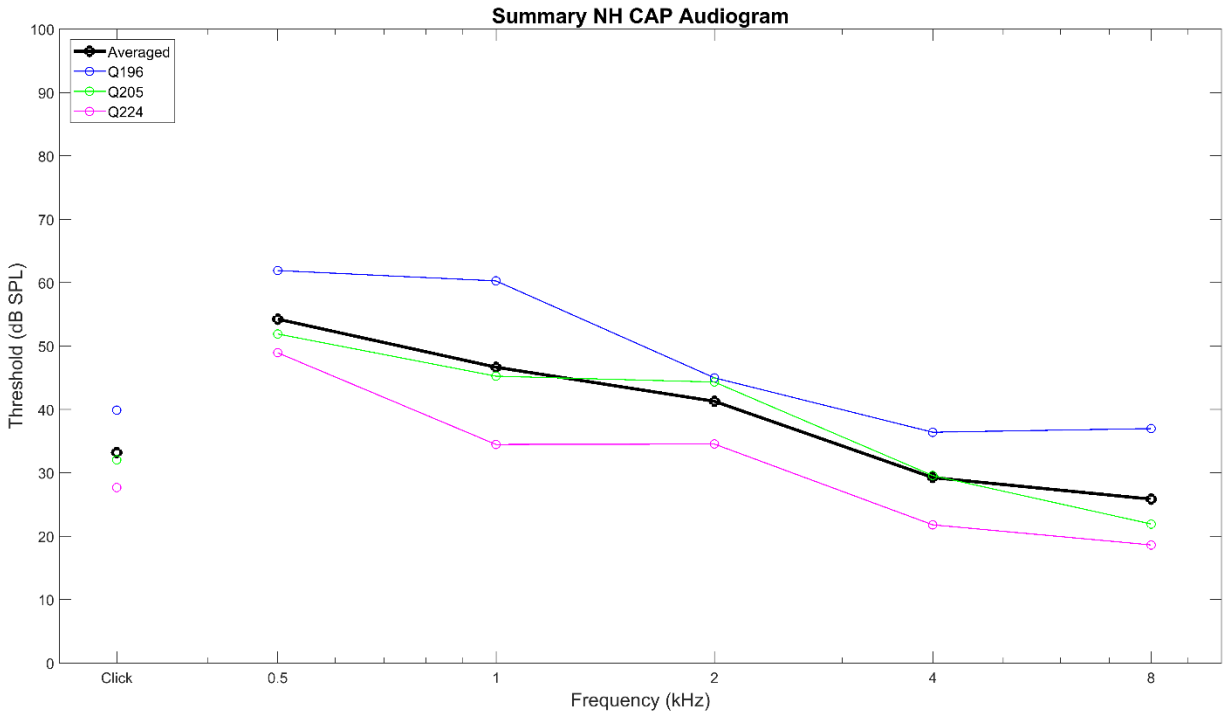

Figure 2.15 Example of an average audiogram for one metric across chinchillas with the same hearing type (NH) for click and tone responses. The different line colors correspond to the different chinchillas, and the thick black line represents the average of these chinchillas.

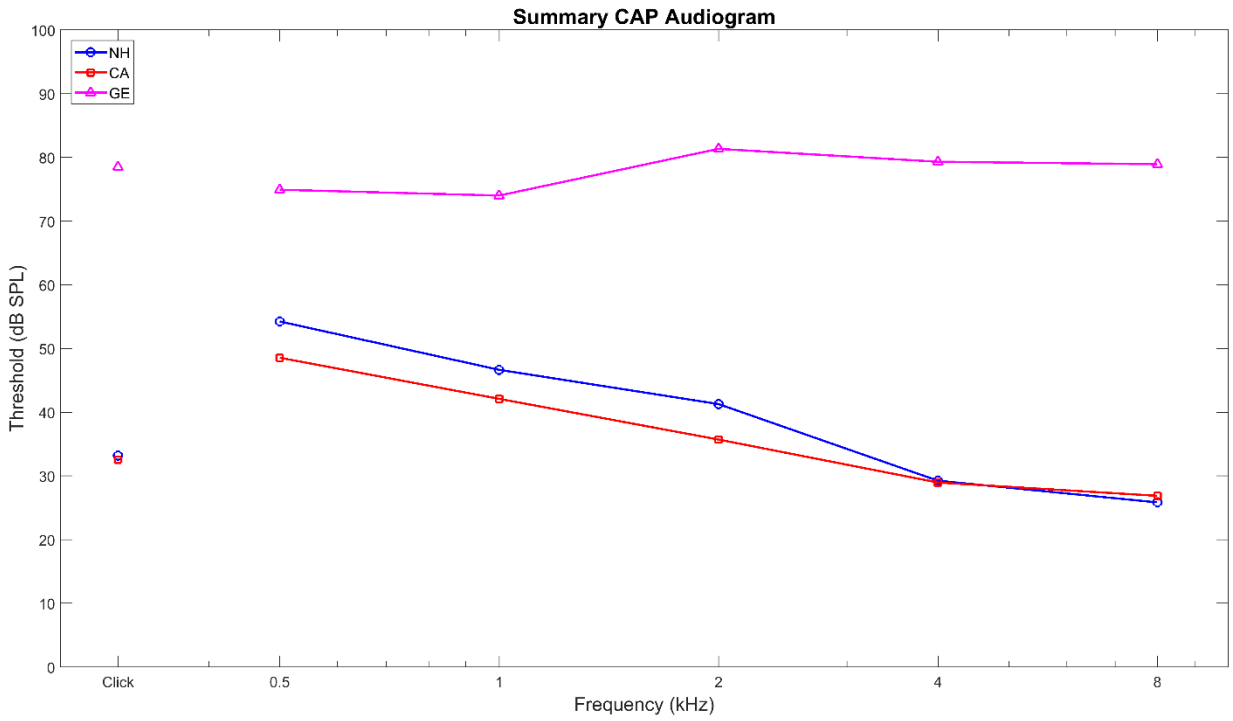

Figure 2.16 Plot comparing the different hearing types for the average audiograms of the CAP metric for click and tone responses. The line colors and symbols correspond to the different hearing types with a legend in the upper left corner.

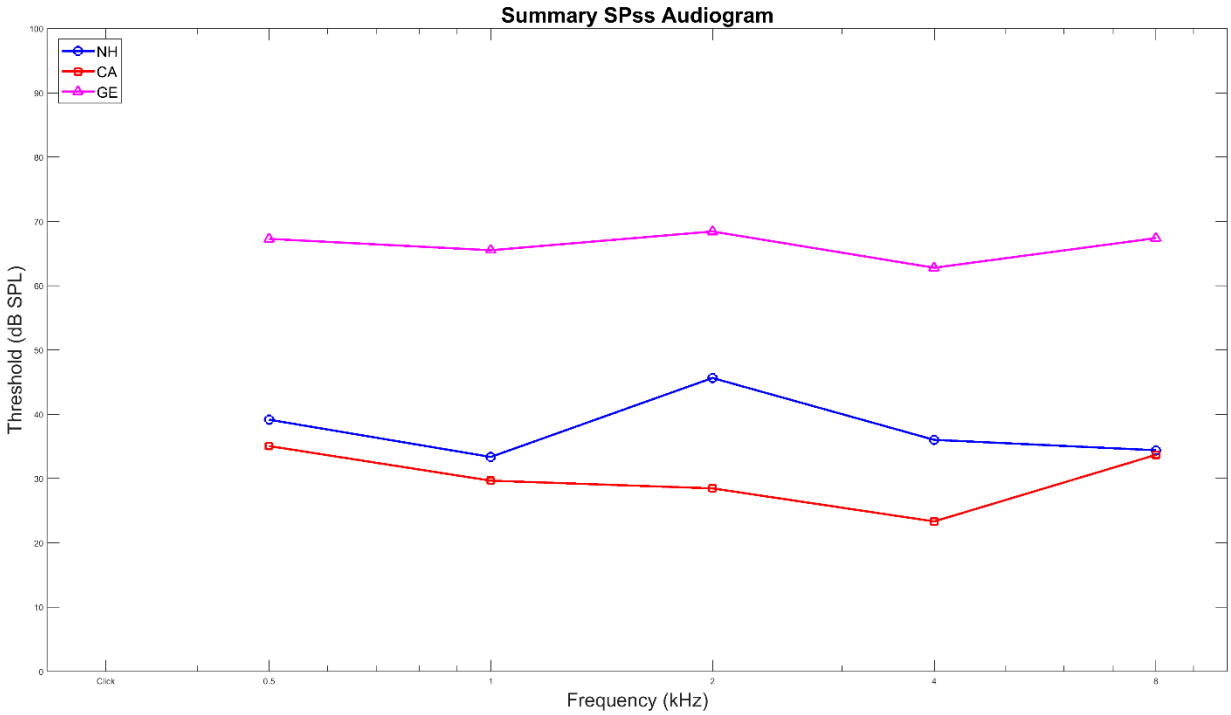

Figure 2.17 Plot comparing the different hearing types for the average audiograms of the SPss metric for click and tone responses. The different line colors and symbols correspond to the different hearing types with a legend in the upper left corner.

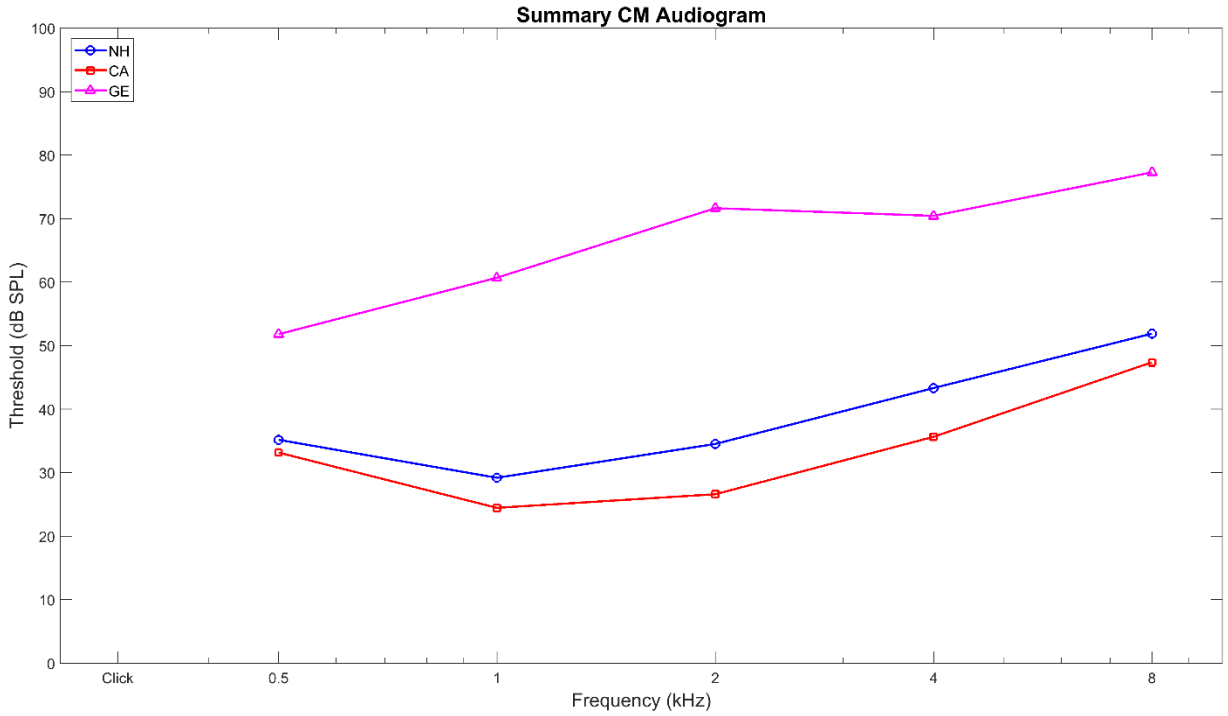

Figure 2.18 Plot comparing the different hearing types for the average audiograms of the CM metric for click and tone responses. The different line colors and symbols correspond to the different hearing types with a legend in the upper left corner.

2.6 Discussion

For the calculation of the individual metrics, the automated process of finding and calculating them worked well for the most part, though it was not perfect, especially for identifying some N1s and P1s for the CAP amplitude. For the NH click responses, the calculation of the metrics worked very well, an example of which is shown in Fig. 2.1 above. For the CA and GE click responses, because some of the response shapes were different, it was more difficult to accurately identify the CAP, examples of which are shown in the appendix below. For the tone responses, especially the higher frequencies like 2 and 4 kHz, if there was any lingering microphonic in the averaged response, it could affect where P1 was defined and change the amplitude of the CAP, an example of which is shown in the appendix. It should be noted that the sampling rate when the data was recorded was around 12.2 kHz, meaning that responses to tones up to 6 kHz can be reliably recorded. Also, as mentioned above, the filtering was 300-3000 Hz, which will reduce the CM response recorded for higher frequencies.

The calculation of thresholds for the three different metrics was consistent across hearing type. For CAP and CM, the calculation of threshold appeared to work well and to be an accurate representation of threshold for the different hearing types. However, because the recording set up for collecting the data was not optimized to capture the SP (300-3000 Hz recordings do not allow DC offsets to come through well), the calculation of threshold for SP was less accurate and less consistent compared to the other two metrics, and as such should be interpreted with caution based on these data.

In terms of identifying hearing loss and potential location, the metrics worked well. From the figures that show the comparison of the three different hearing types for the different measures versus level, it is clear to see that the CA hearing type generally followed the NH trend, though usually with a slightly smaller response, whereas the GE hearing type had significantly shifted higher thresholds for all three measures. This shift in threshold is confirmed in the average audiogram figures above that compare the different hearing types where GE consistently has a higher threshold compared to the other two across frequency. These audiometric results are consistent with ABR and AN-fiber thresholds measures from these animals [18].These results are also consistent with the specific damage confirmed from the original data collection where the GE chinchillas had significant OHC loss and the CA chinchillas had only mild IHC loss, as shown in Fig. 2.19 below [18].

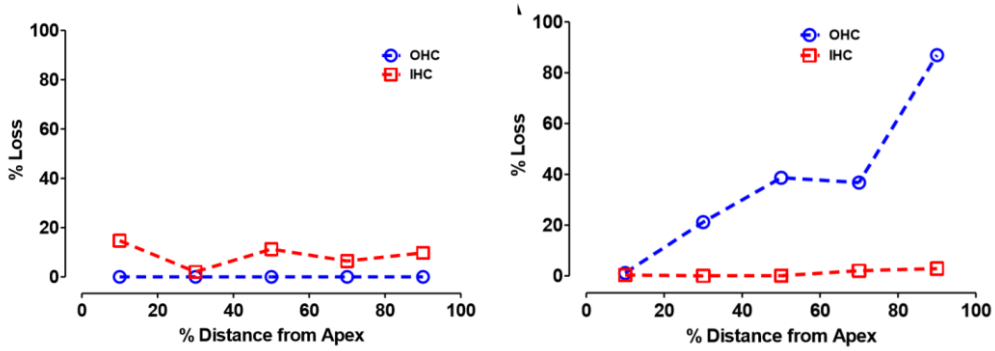

Figure 2.19 Quantified hair cell loss for CA and GE chinchillas [18]. The left graph shows the hair cell loss for CA chinchillas who had mild IHC loss and no OHC loss. The right graph shows the hair cell loss for GE chinchillas who had significant OHC loss, especially at higher frequencies, and mild to no IHC loss.

3. FEASIBILITY OF NON-INVASIVE ELECTROCOCHLEOGRAPHY FROM THE EAR CANAL OF AWAKE CHINCHILLAS

3.1 Recording Setup

The electrocochleography responses were recorded from gold-foil tiptrodes placed in the ear canal of either anesthetized or awake chinchillas. The output of the tiptrodes was first passed through a voltage follower that was powered and grounded to a preamplifier. The output of the voltage follower went to the same preamplifier where the signal was filtered and amplified and then passed to the A-D converter in the TDT recording module.

3.1.1 Stimuli

Similar to the stimuli used in the previous chapter, the stimuli used here to elicit the ECochG responses were either clicks or tone bursts, both of which were presented with alternating polarities. The clicks were 0.04 ms in duration with an initial period of 50 ms for the anesthetized recordings that was changed to 43 ms for the awake recordings in order to help reduce power line noise. Since the signal was being recorded from the ear canal which is further from the source than the round window, the number of polarity pairs presented was increased. For the anesthetized recordings, 300 sets of each polarity were presented while the awake recordings used either 300, 2,000, or 3,000 sets during this preliminary data collection. The tone bursts were 10 ms in duration again with an initial period of 50 ms that was changed to 43 ms. They were presented at either 0.5, 1, 2, 4, or 8 kHz. As with the clicks, the anesthetized recordings used 300 sets of each polarity while the awake recordings used either 300, 2,000, or 3,000 sets. Both the clicks and tones were presented at varying levels of attenuation from the max sound level in dB SPL.

3.1.2 Electrically Shielded Acoustic Transducers

In order to reduce noise and interference from the acoustic transducers that present the sounds, the transducers were placed in electrically shielded boxes. The transducers were wrapped in custom shielding using a combination of metallic tape and metal techflex in order to attenuate electromagnetic artifacts [23] and then placed in and grounded to the electrically shielded boxes.

An example of one is shown in the figure below. For both the anesthetized and awake procedures, Etymotic ER-1 transducers were used.

Figure 3.1 Example of the acoustic transducers in the electrically shielded boxes. The transducer is wrapped in shielding tape and is grounded to the box.

3.1.3 Gold Foil Tiptrodes

To record the responses to the stimuli non-invasively, gold-foil tiptrodes were placed in the ear canal. These electrodes have an earphone foam tip that is wrapped in gold foil. The gold foil is what allows the signal to be recorded, meaning there needs to be good connection between the foil and the ear canal. For the chinchillas, the 10 mm diameter tiptrodes were used. One tiptrode was placed in each ear canal, but sound was only presented to the right ear. Tiptrodes were replaced for each animal, and sometimes within animals if the recordings were too noisy from the foil being rubbed off on difficult insertions (this was only required occasionally).

Figure 3.2 Picture of gold-foil tiptrodes.

3.1.4 Voltage Follower

To help reduce noise and interference, the output of the electrodes was first passed through a voltage follower (buffer amplifier with unity voltage gain), which helps with the higher-impedance tiptrodes. The voltage follower consisted of an operation amplifier (op amp) that was powered by the pre-amplifier. The ground from the pre-amplifier and the ground from a sub-dermal needle electrode on the nose of the chinchilla were connected so there was a common ground. The inputs from the two tiptrodes were connected to the op amp with the ground from the animal's nose, and the two outputs from the op amp were the outputs of the voltage follower that were then connected to the pre-amplifier.

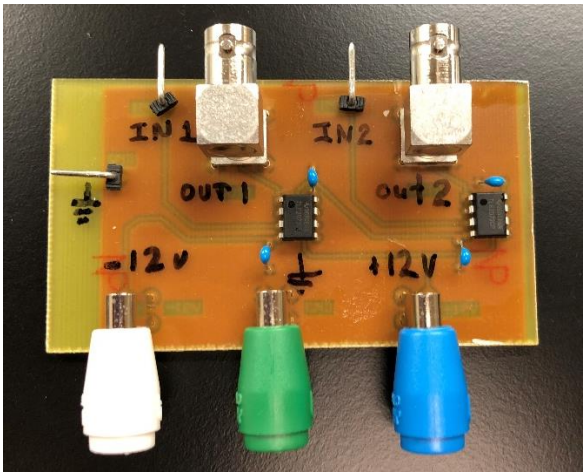

Figure 3.3 Example of the voltage follower used to help reduce noise and interference. This is the first version of the voltage follower which shows how the inputs and outputs for the two tiptrodes are connected to the op amps and powered by the pre-amplifier. IN 1 is the input from the right tiptrode, IN 2 is the input from the left tiptrode, OUT 1 is the output from the op amp for the right tiptrode, OUT 2 is the output from the other op amp for the left tiptrode. The ground pin on the left of the board is the input from the ground subdermal needle electrode. The three inputs along the bottom of the board are the power inputs from the pre-amplifier to power the op amps.

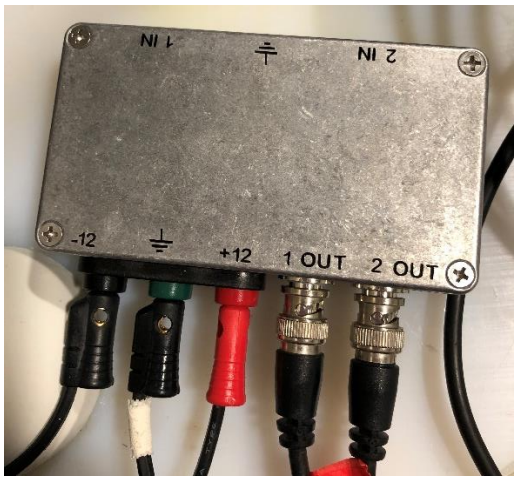

Figure 3.4 This is the updated, enclosed version of the voltage follower with better streamlining of inputs and outputs. This final version was the one used during all of the anesthetized and awake experiments.

3.1.5 Low Noise Voltage Preamplifier (SR 560)

The pre-amplifier used for the ECochG recordings was the SR 560. One of the reasons this pre-amplifier was used was because it has an option to connect two inputs which are then subtracted in an A – B configuration. The output of the right tiptrode (ipsilateral) was connected to the A input and the output of the left tiptrode (contralateral) was connected to the B input. Because the left ear did not receive any sound, any signal from the ear would be noise which could then be subtracted from the signal coming from the right ear that does receive sound.

The SR 560 also provided filtering and gain to the response signal. Another reason this pre-amplifier was chosen was because of its ability to pass a near-DC response in the filtering. This is important because the SP portion of the ECochG is a DC offset in the signal. The high pass cutoff for the filter changed between 0.3, 3, and 30 Hz, but the low pass cutoff was always 3 kHz. The gain provided to the signal was always 50,000 X (the highest option on the SR 560; occasionally this highest gain setting overloaded the SR 560, in which cases noise was reduced by repositioning the electrodes before recording). The low-noise setting on the SR 560 was used, and the 50 ohm output was always used.

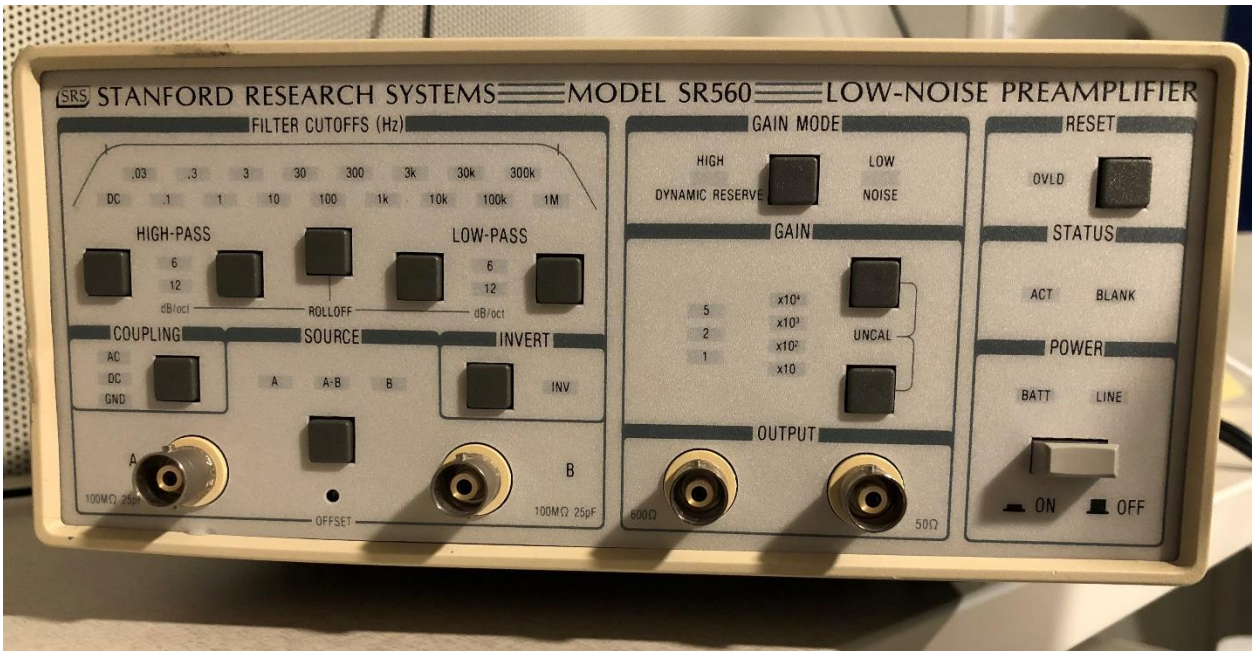

Figure 3.5 Image of the front of the SR 560 pre-amplifier.

3.2 Initial Anesthetized Results

The use of non-invasive gold-foil tiptrodes placed in the ear canal to record ECochG responses was first tested in anesthetized chinchillas in order to work out and refine recording parameters, as well as to ensure that responses were comparable to those recorded at the round window before attempting to record from awake animals where there is more noise. Since these were primarily feasibility studies, there are more single-example figures shown in this chapter and fewer summary figures given the scope that was possible with the COVID-19 pandemic.

Three anesthetized procedures were performed on the same chinchilla over the course of a couple of months (post COVID-19 lab re-opening). The chinchilla was anesthetized with xylazine (4.0 mg/kg, SQ) followed by ketamine (40 mg/kg, SQ) at doses that allowed for about 2 hours of recording. Heart rate and blood oxygen levels were monitored throughout the procedure, and the body temperature was maintained around 37 ℃ with a heating blanket placed underneath the animal. Ringers was given subcutaneously (6 cc before recordings and 6 cc after). All animal procedures were conducted using an approved PACUC protocol (#1111000123).

During the first procedure there was significant 60 Hz noise which was eventually reduced by braiding the three recording electrode cables around each other (two tiptrodes and subdermal ground). The change in noise is shown in Fig. 3.6 below. Once the noise was greatly reduced (but

not eliminated, as seen in the subsequent figures), responses to clicks were recorded at varying levels of attenuation ranging from 0 – 100 dB with filtering of 30 – 3000 Hz and 300 sets of each polarity. After recording responses from the ear canal, we recorded the acoustic click response coming out of the tiptrode (on the table in open air) in order to compare onset of the stimulus with the ECochG responses. The initial figures for these responses are shown in Figs. 3.7-3.9 below, where despite some remnant 60 Hz noise, a clear set of ECochG waves following stimulus onset are observed.

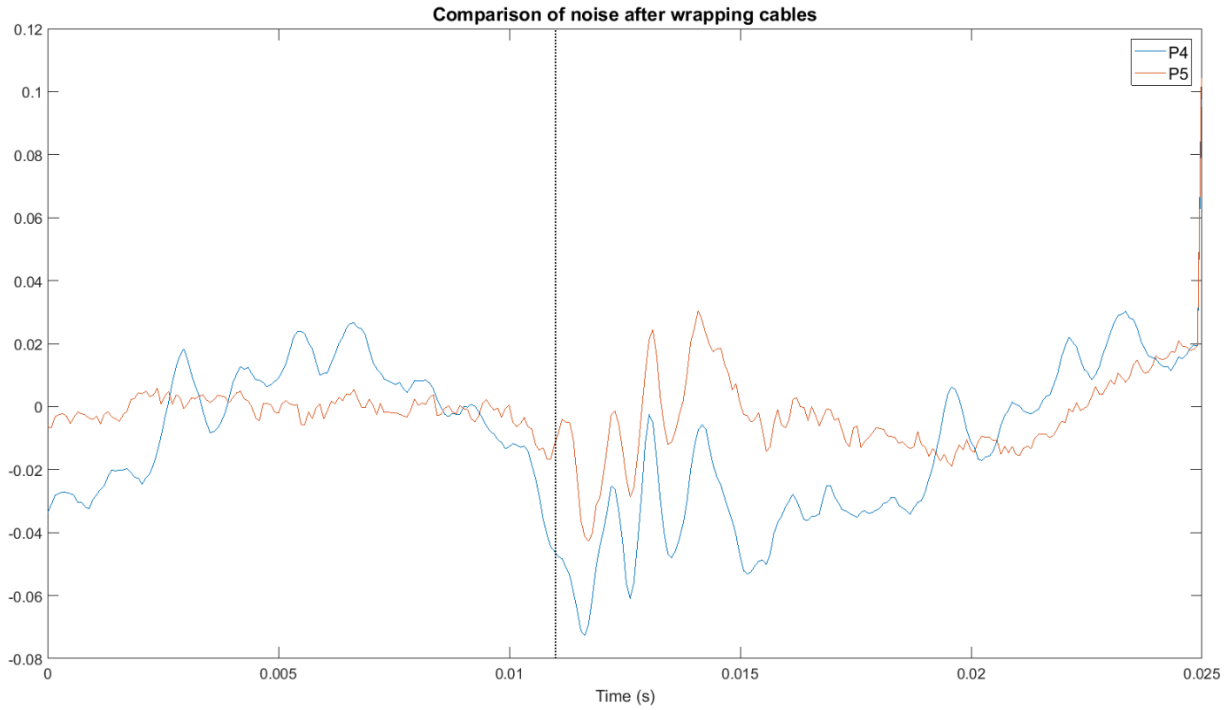

Figure 3.6 Comparison of noise after wrapping electrode cables around each other. P4 was the response to a click before the cables were wrapped (significant 60 Hz oscillations), and P5 was the response to a click after wrapping the cables. Both responses had the same filter width and attenuation. Note: in our custom software for recording, P4 refers to "picture #4", i.e., data file #4. Both files were baseline corrected to 0 where the baseline region was 0 to 11 ms (indicated by the dotted black line representing rough stimulus onset).

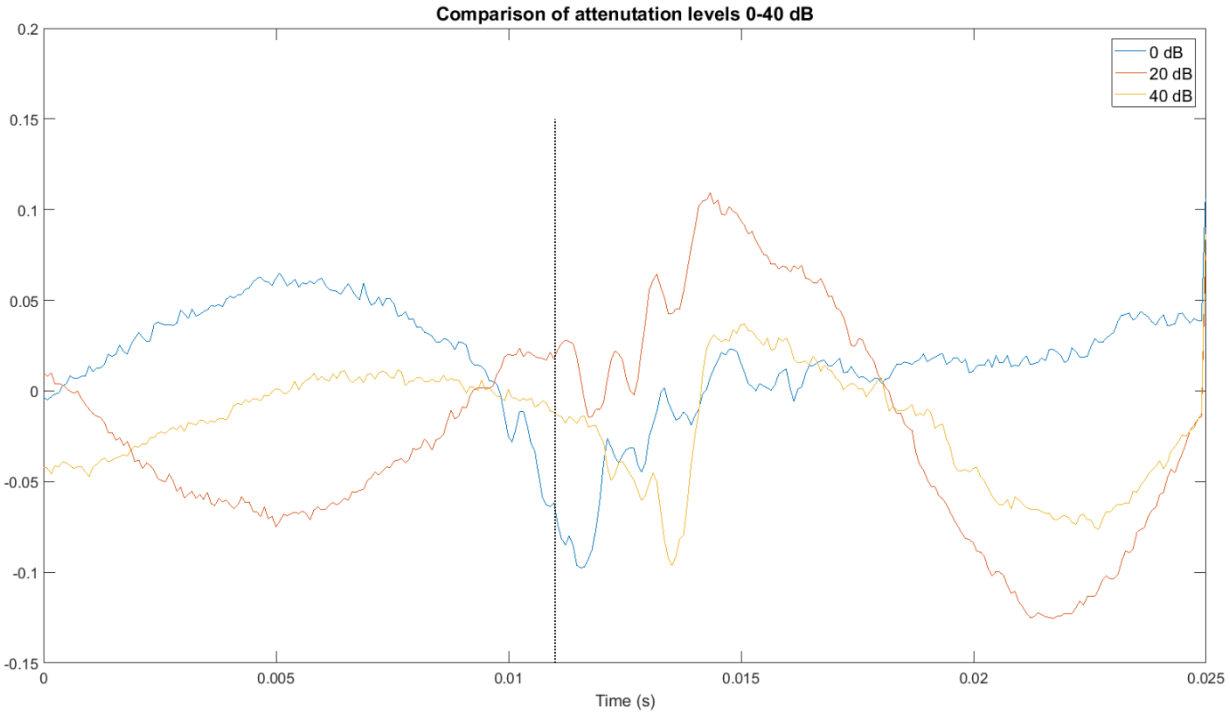

Figure 3.7 Comparison of ECochG responses to clicks at attenuation levels ranging from 0 to 40 dB. The vertical dotted black line represents the approximate onset of the click stimulus. The responses have been individually baseline corrected where the baseline window is from 8-11 ms. Note that despite cables being braided, significant 60 Hz noise was sometimes still observed. Subsequent figures illustrate several additional approaches to removing remaining 60 Hz noise.

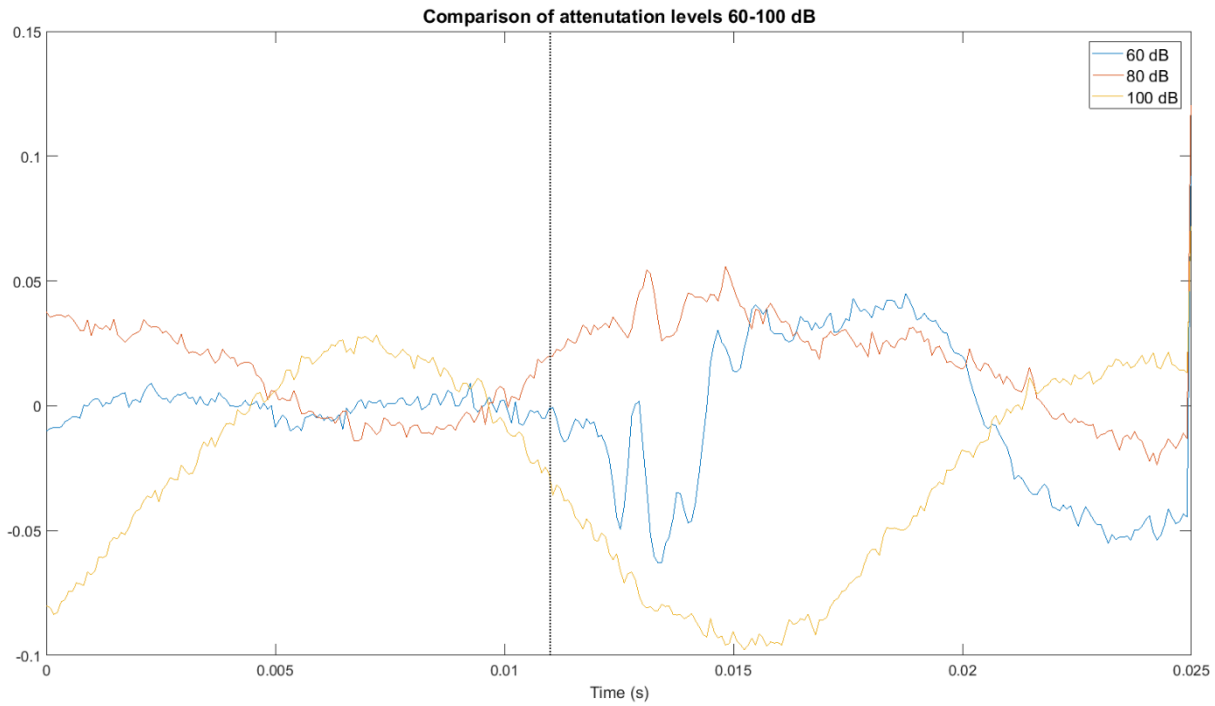

Figure 3.8 Comparison of ECochG responses to clicks at attenuation levels ranging from 60 to 100 dB. The vertical black line represents the approximate onset of the click stimulus. The responses have been individually baseline corrected where the baseline window is from 8-11 ms. Note that click threshold is between 80 and 100 dB attenuation, which is in line with typical click thresholds in our lab.

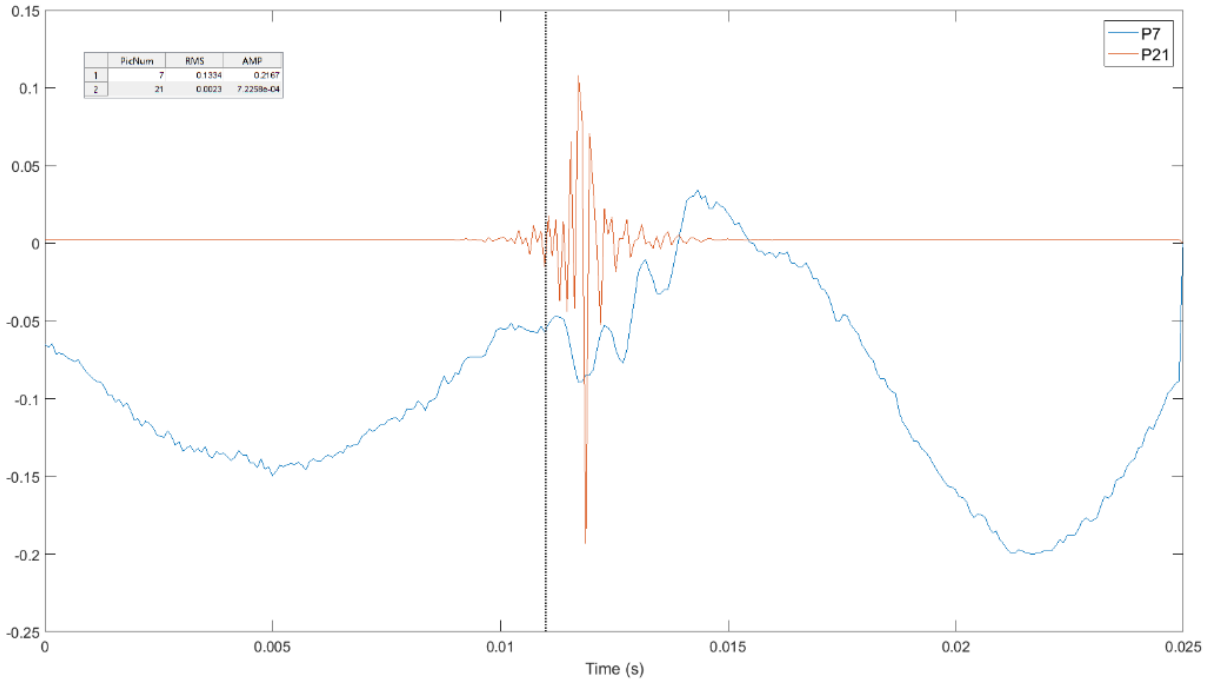

Figure 3.9 Comparison of the acoustic click response and an ECochG response from a tiptrode. P21 is the acoustic click and P7 is the click response at 20 dB attenuation.

The second procedure corrected for lingering 60 Hz noise due to 50 ms periods (integer multiple of 60 Hz period) in the stimuli repetition by changing to a 43 ms period. After the noise due to the period was reduced, as shown in Fig. 3.10 below, a major focus of this procedure was trying different filter parameters. A comparison of responses with the different filter parameters is shown in Fig. 3.11 below. There was still some lingering noise and variability in the responses, especially before and after the ECochG response, so a notch filter with one magnitude-one zero pair at 60 Hz, and a magnitude-0.95 pole pair at 60 Hz to sharpen the notch was applied to the responses to try to reduce this noise which greatly helped, as seen in Fig. 3.12 below. After trying different filter widths, we collected responses to clicks from a full range of attenuation levels that spanned from 20 to 95 dB in 5 dB steps. Comparisons of the filtered responses at different attenuation levels are shown in Figs. 3.13 and 3.14 below. It should be noted that during this procedure, sound was accidentally presented to both ears instead of just to the right ear, and the sound presented to the left ear was not delayed by the calibration filter compensation, and this accounts for the early peaks before the onset of the stimulus to the right ear.

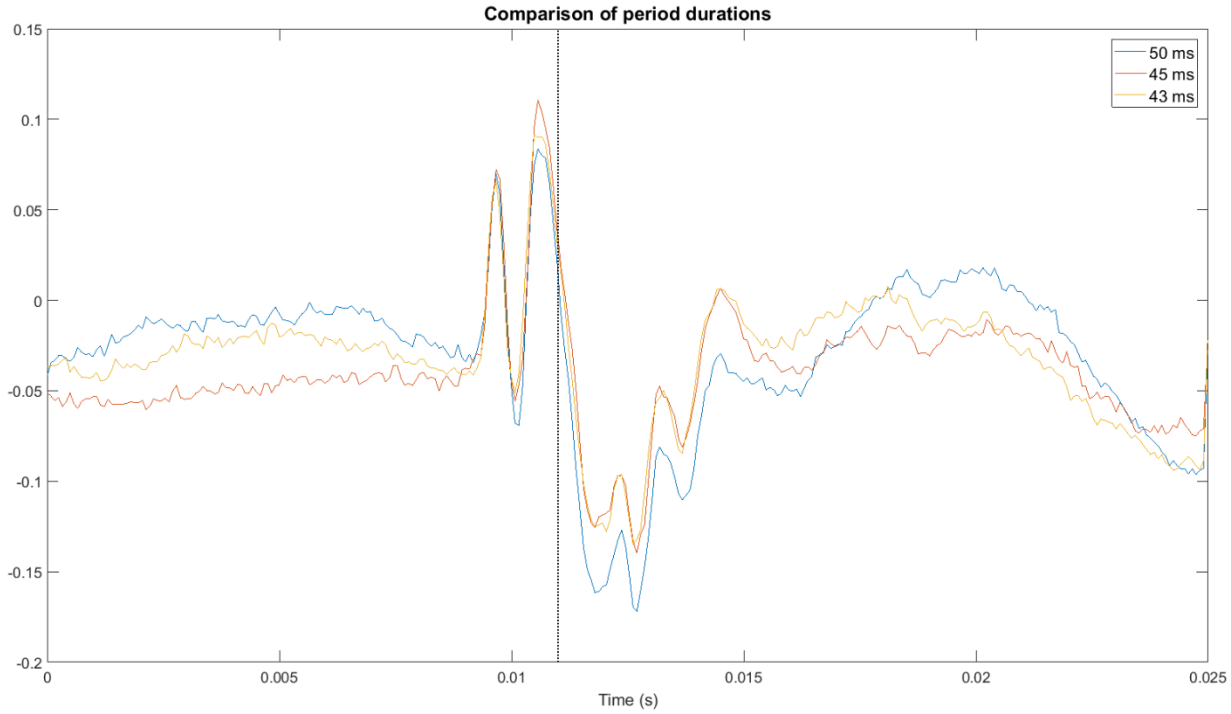

Figure 3.10 Comparison of lingering noise with different period durations. Note that during this procedure, sound was accidentally presented to both ears, and the left ear was not delayed by the calibration filter compensation, which accounts for the early peaks before the onset of the stimulus to the right ear. The legend in the upper right corner indicates which lines correspond to which period durations. Note that the original 50 ms period is an integer multiple of the period of 60 Hz, whereas 45 and 43 ms are not so that the remnant 60 Hz noise cancels across repetitions – those curves are flatter pre and post ECochG response. The vertical black dotted line represents the approximate onset of the click stimulus to the right ear. The responses have been individually baseline corrected.

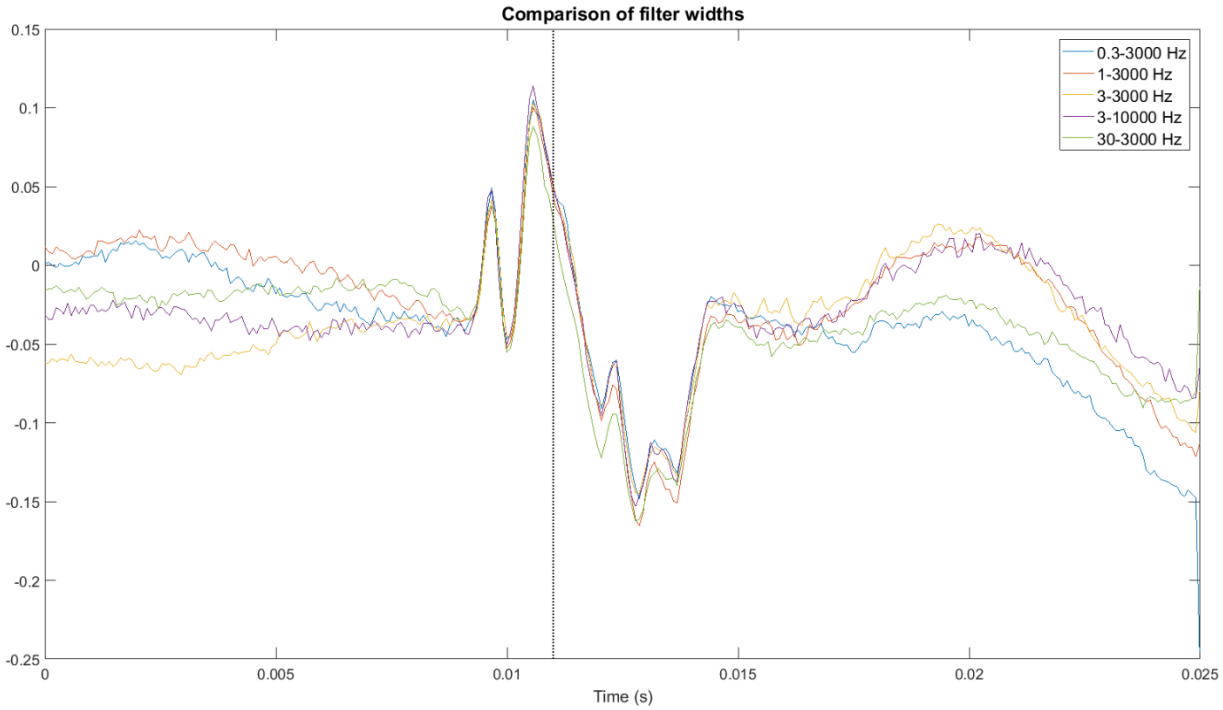

Figure 3.11 Comparison of ECochG responses with different filter widths on the SR 560. Note that during this procedure, sound was accidentally presented to both ears, and the left ear was not delayed by the calibration filter compensation, which accounts for the early peaks before the onset of the stimulus to the right ear. However, the point of this figure is to show filter differences. The legend in the upper right corner indicates which lines correspond to which filter widths in Hz. The vertical black dotted line represents the approximate onset of the click stimulus to the right ear. Note the remnant 60 Hz noise that still remains. The next figure shows that a simple notch filter can remove this artifact.

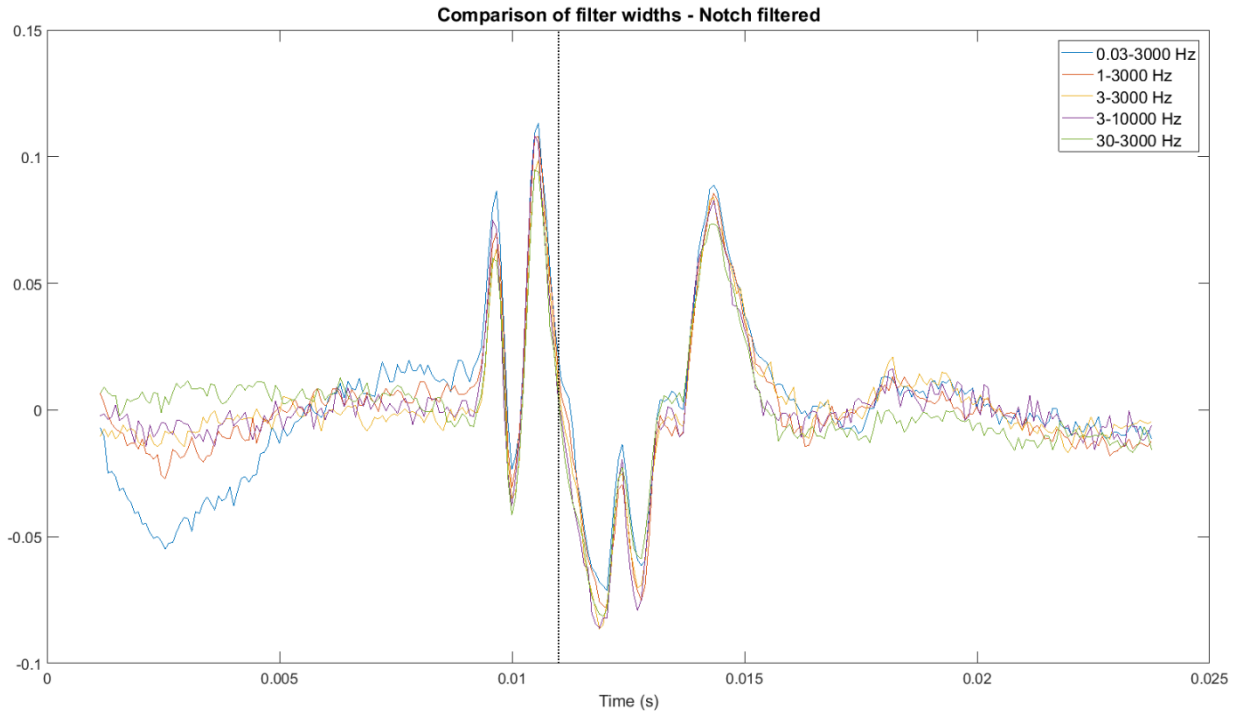

Figure 3.12 Comparison of the different filter width responses after a notch filter at 60 Hz was applied. The legend indicates which line colors correspond to which filter widths. The vertical black line represents the approximate onset of the click stimulus to the right ear. Note that the notch filter (one magnitude-one zero pair at 60 Hz, and magnitude-0.95 pole pair at 60 Hz to sharpen the notch) generally flattens the pre- and post-ECochG responses quite well.

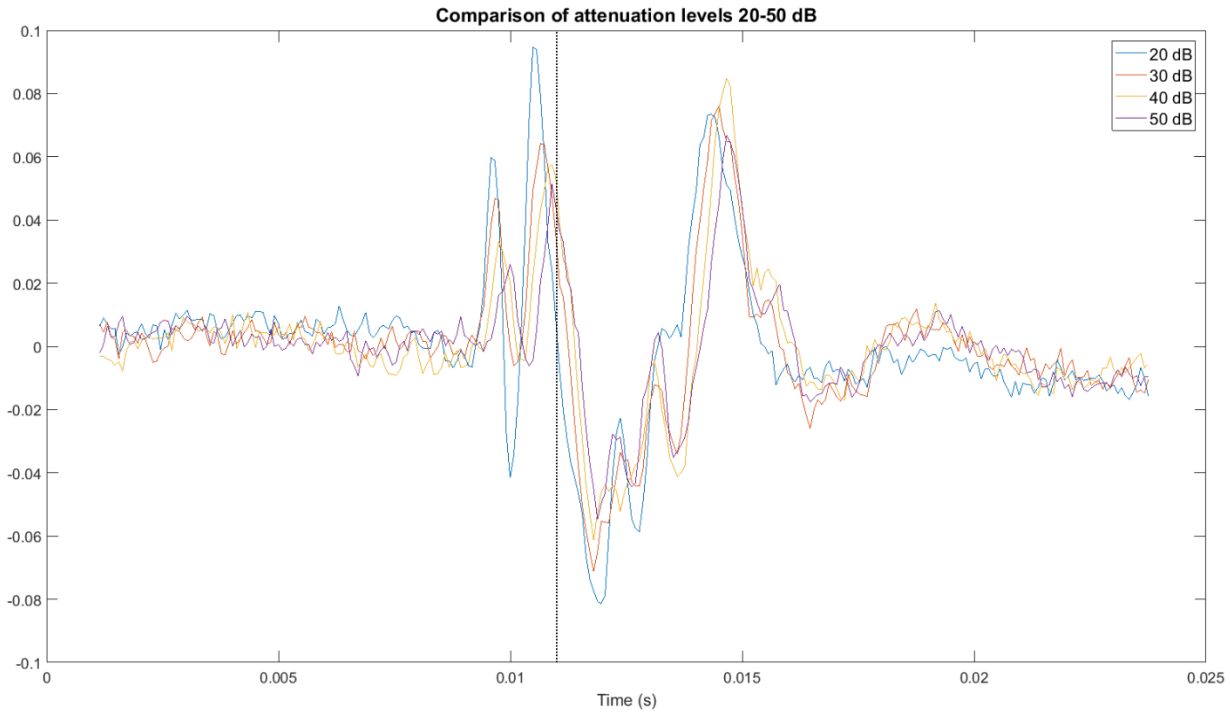

Figure 3.13 Comparison of notch filtered ECochG responses to clicks at attenuation levels ranging from 20 to 50 dB. The legend in the upper right corner indicates which lines correspond to which attenuation levels. The vertical black line represents the approximate onset of the click stimulus to the right ear. Note that all 4 levels are well above threshold, whereas the next figure shows threshold is at lower sound levels. Also note that a click stimulus was presented to both ears and the left ear was not delayed.

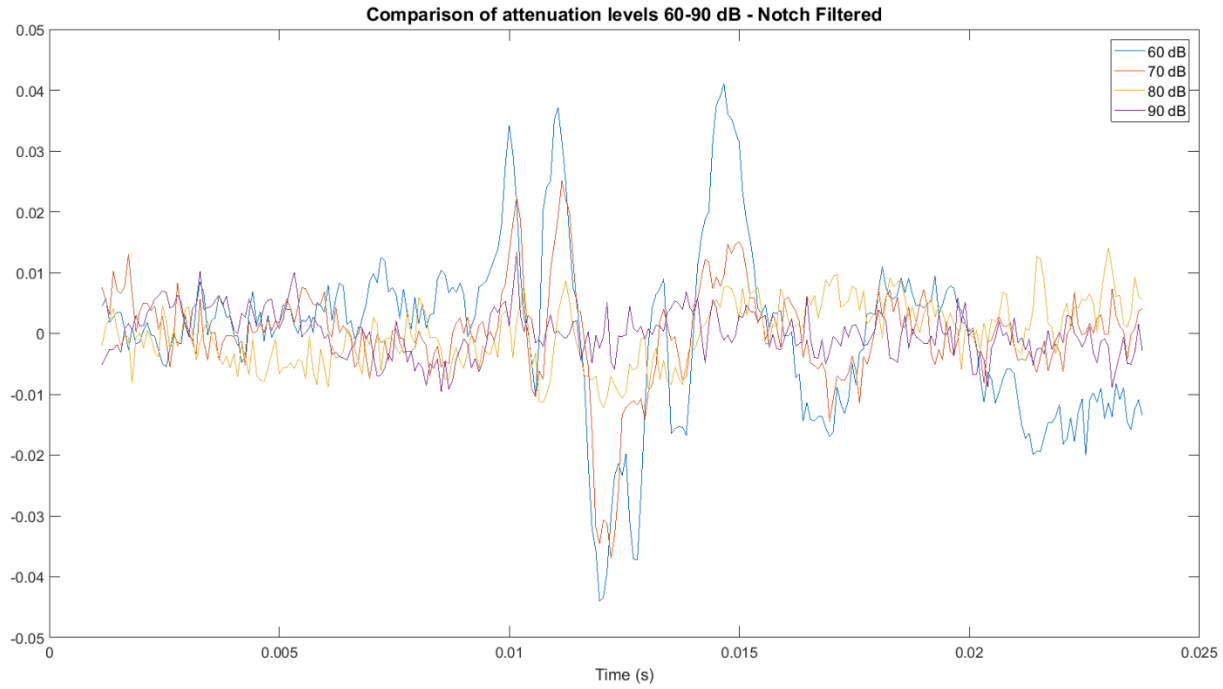

Figure 3.14 Comparison of notch filtered ECochG responses to clicks at attenuation levels ranging from 60 to 90 dB. The legend in the upper right corner indicates which lines correspond with which attenuation levels. Note that threshold is around 80 dB attenuation which is in line with typical click thresholds in our lab. Also note that a click stimulus was presented to both ears and the left ear was not delayed.

During the third and final anesthetized procedure, we were able to record calibrations of sound level inside the ear canal with a probe microphone inserted into the tiptrode (note that the tiptrode does not have a microphone itself). In order to do this, a hot drill bit was used to make a small hole in the foam portion of the tiptrode parallel to the acoustic tube. This hole was just large enough for the flexible ER-7C probe mic to be inserted into the hole and glued to the tiptrode such that the open end of the mic probe tube aligned with the end of the tiptrode. The tiptrode with the probe mic attached was then placed in the ear canal and calibrations were recorded which are shown in Figs. 3.15 and 3.16 below. Because we were only able to record decent calibrations during this one procedure since our focus was on ECochG feasibility, we have left levels in terms of attenuation rather than converting to sound level in dB SPL in all figures, but since we used the same equipment for all procedures, these calibrations can give rough estimates of the sound level in dB SPL. To approximate the sound level for tone responses, subtract the attenuation level from the maximum sound level of the calibration (Fig. 3.15) at that same frequency. To approximate

the sound level for click responses, subtract the attenuation level from the average sound level of the inverse calibration (Fig. 3.16) up to 10 kHz.

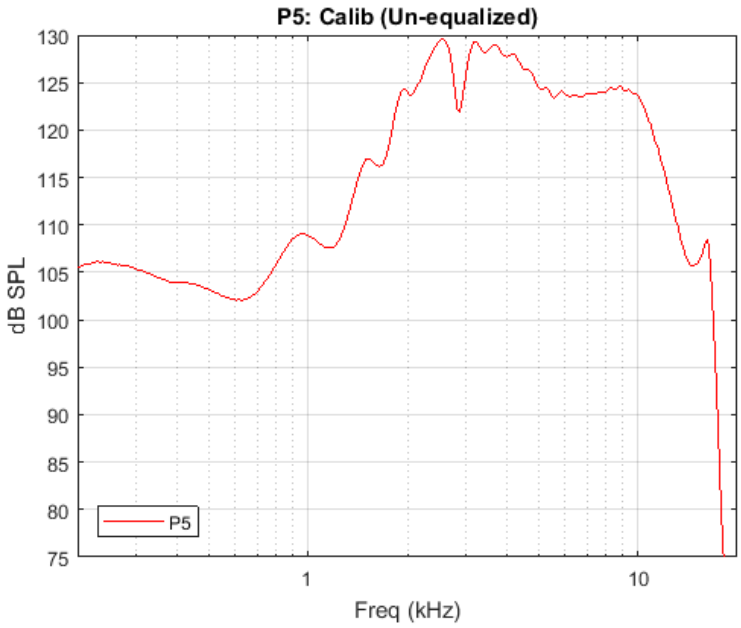

Figure 3.15 Raw calibration response from a probe microphone inside a tiptrode placed in the ear canal. This compensated-calibration curve was used for all subsequent experiments with tone stimuli due to the difficulty in placing the probe tube and the focus on ECochG feasibility. To approximate sound level in dB SPL for tone responses, subtract the attenuation level from the maximum sound level of the calibration at that frequency. For a 2 kHz tone response at 20 dB attenuation, the maximum sound level at 2 kHz is about 125 dB SPL on the calibration, so the approximate sound level of the response is 105 dB SPL. Due to the non-flat nature of the ER-1 response in a chinchilla ear canal, we used an inverse FIR filter to compensate for this calibration curve, as shown in the next figure.

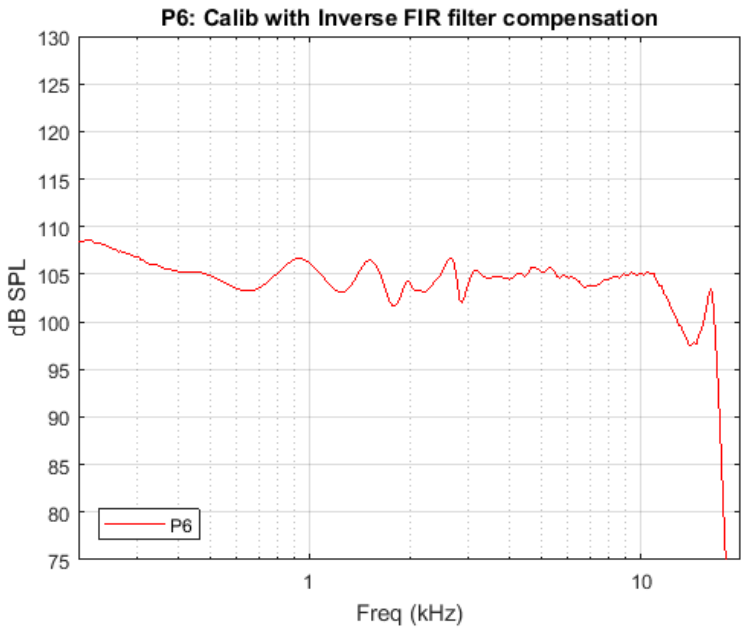

Figure 3.16 Inverse calibration response from a probe microphone inside a tiptrode placed in the ear canal. This compensated calibration curve was used for all subsequent experiments with click stimuli due to the difficulty in placing the probe tube and the focus on ECochG feasibility. To approximate sound level in dB SPL for click responses, subtract the attenuation level from the average sound level of the calibration up to 10 kHz. For a click response at 20 dB attenuation, the average sound level is about 105 dB SPL on the calibration, so the approximate sound level of the response is 85 dB SPL.

After calibrating, the final anesthetized procedure again tried different filter widths, but this time in response to a 2 kHz tone burst. These responses were also notch filtered, and the comparison of the different filter width responses is shown in Fig. 3.17 below, and it should be noted that these particular responses were collected with a fixed phase to illustrate the sustained oscillatory response associated with the CM. After this we again collected responses to clicks at varying attenuation levels in order to compare results from the previous procedure. Attenuation levels ranged from 20 to 85 dB in 5 dB steps with a filter width of 0.03 – 3000 Hz. Comparisons of the different attenuation levels are shown in Figs. 3.18 and 3.19 below, and the approximate threshold observed (~70 dB attenuation) is at an expected level based on previous CAP responses in our chinchillas. We finally also collected responses to a 2 kHz tone burst at varying levels of attenuation that ranged from 20 to 95 dB in 5 dB steps. Comparisons of the different levels are shown in Figs. 3.20 and 3.21 below.

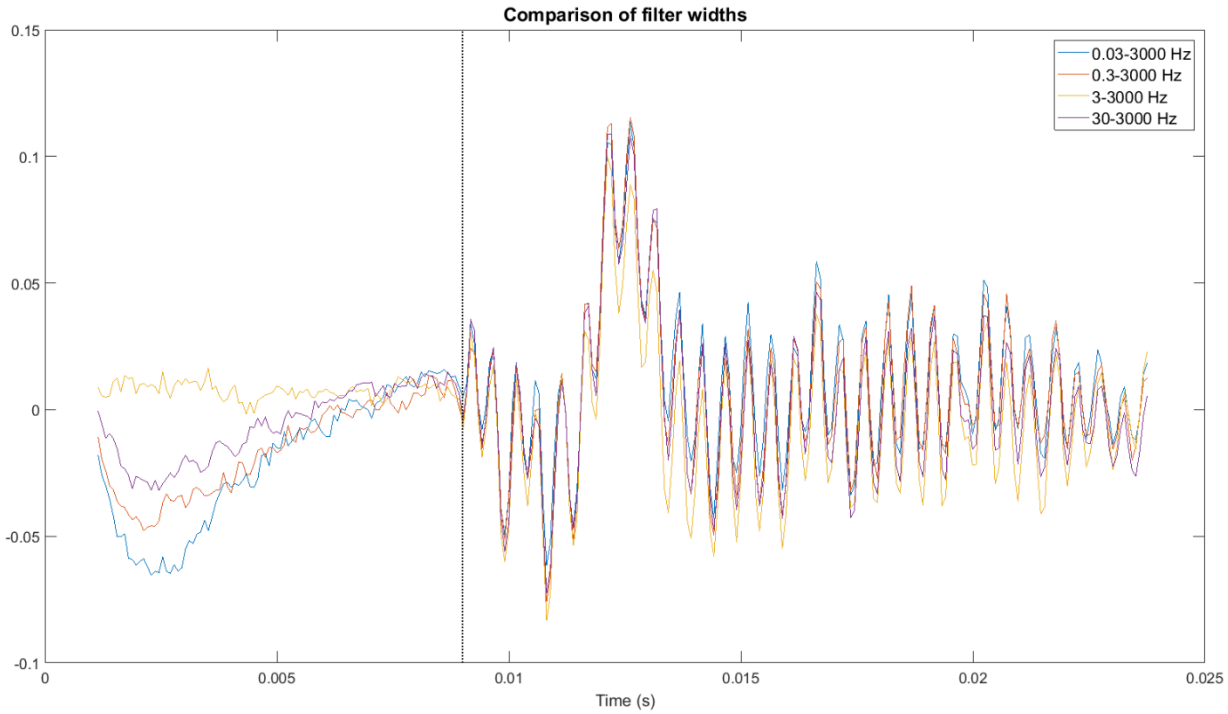

Figure 3.17 Comparison of filtered ECochG responses to a fixed phase 2 kHz tone with different filter widths on the SR 560. The legend in the upper right corner indicates which lines correspond to which filter widths. The vertical black line represents the approximate onset of the tone stimulus. Responses have been notch filtered.

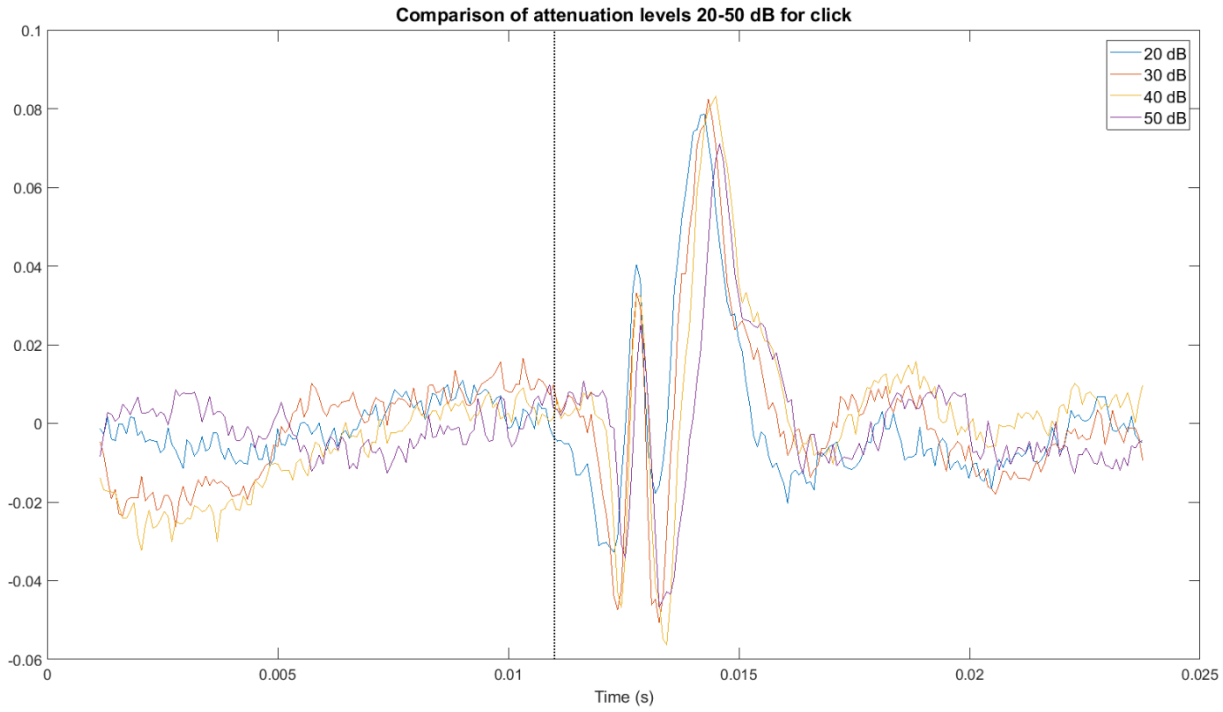

Figure 3.18 Comparison of click responses at louder attenuation levels. The vertical dotted black line indicates the approximate onset of the click stimulus. The line colors correspond to different attenuation levels, and a legend is given in the upper right corner. A filter width of 0.03-3000 Hz was used. Responses have been notch filtered. Note that the loudest sound level (20 dB attenuation, light blue line) has a reduced N1 amplitude compared to the other sound levels, perhaps due to saturation of some sort.

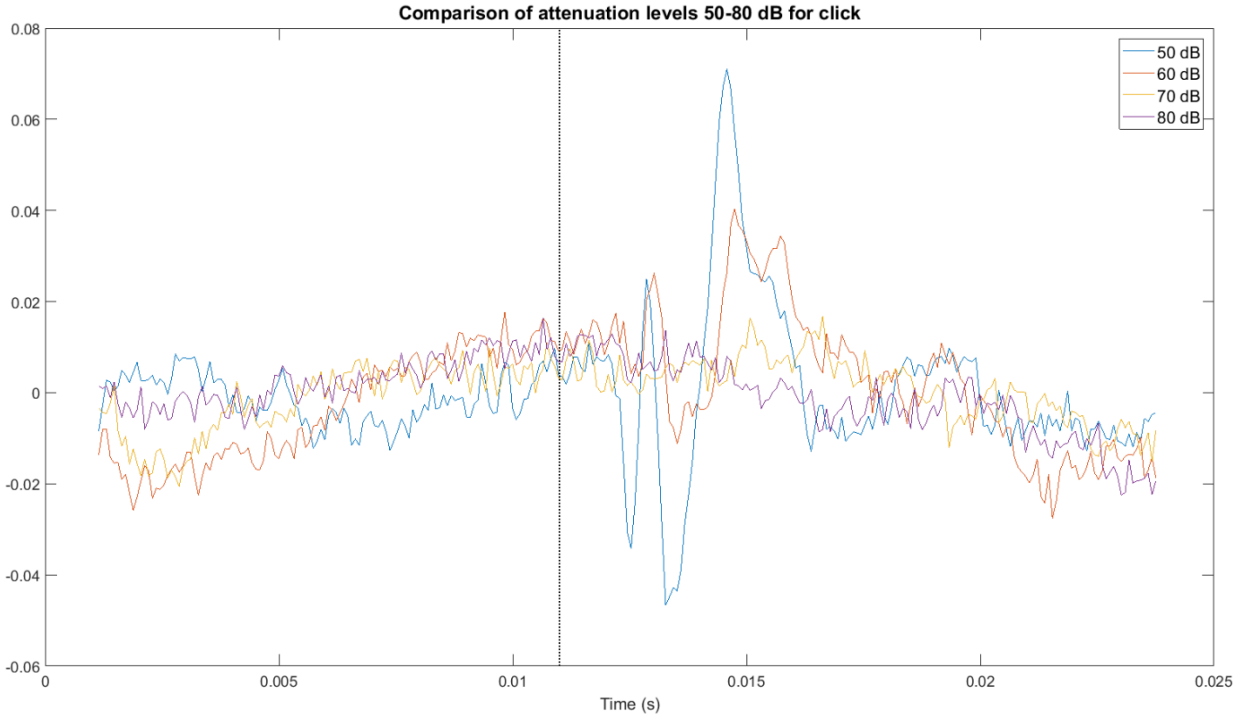

Figure 3.19 Comparison of click responses at attenuation levels approaching threshold. The vertical dotted black line indicates the approximate onset of the click stimulus. The line colors correspond to different attenuation levels and a legend is provided in the upper right corner. A filter width of 0.03-3000 Hz was used. Responses have been notch filtered.

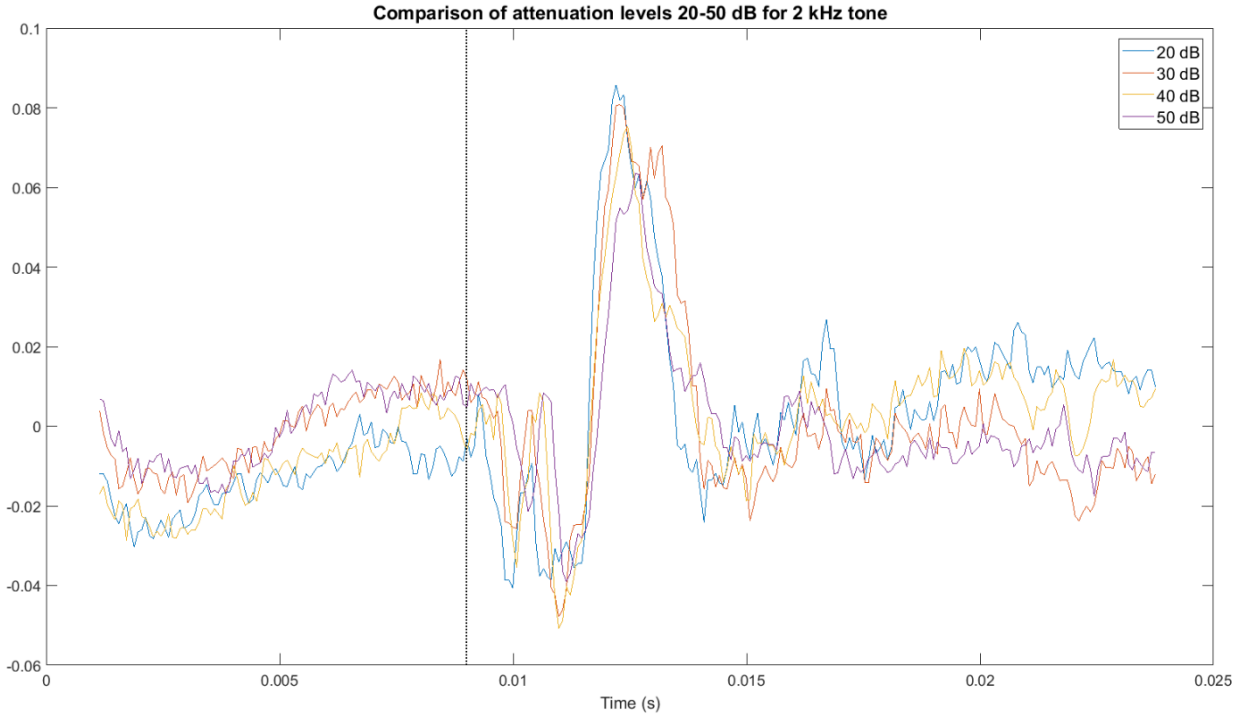

Figure 3.20 Comparison of responses to a 2 kHz tone burst at varying, relatively loud, attenuation levels. The vertical dotted black line represents the approximate onset of the tone stimulus. The line colors correspond to the different attenuation levels. A filter width of 0.03-3000 Hz was used. Responses have been notch filtered.

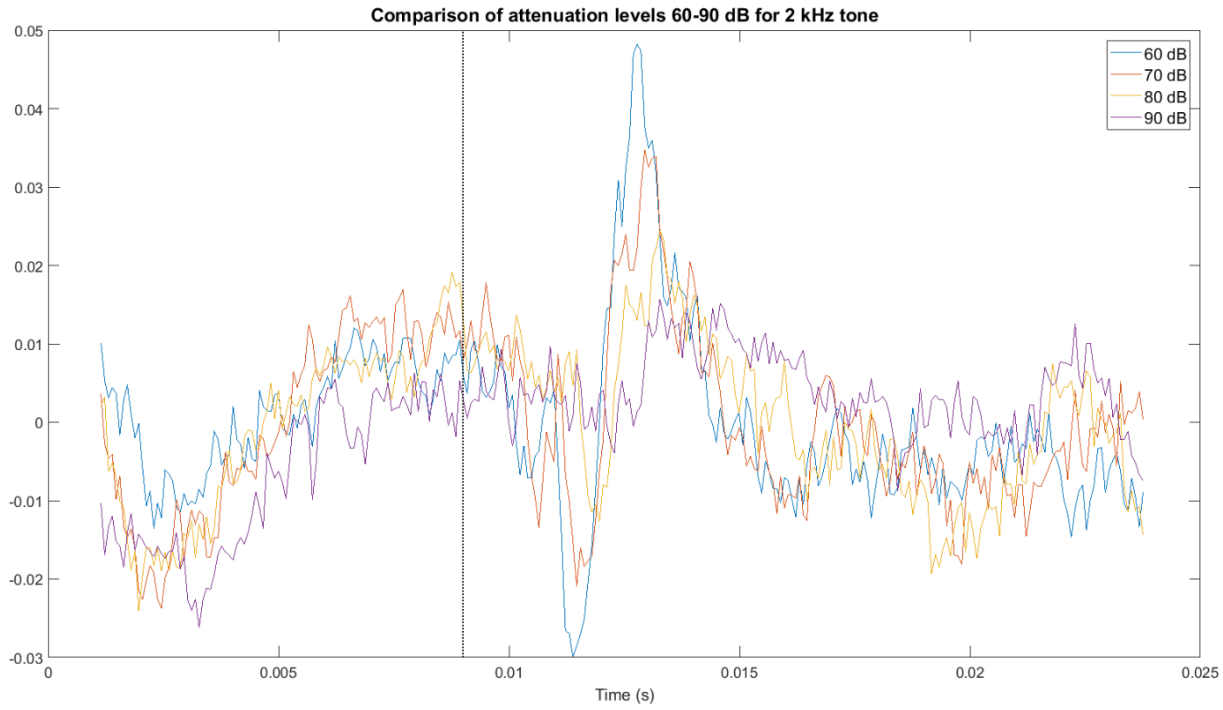

Figure 3.21 Comparison of responses to a 2 kHz tone burst at attenuation levels nearing threshold. The vertical black line represents the approximate onset of the tone stimulus. The line colors correspond to different attenuation levels. A filter width of 0.03-3000 Hz was used. Responses have been notch filtered. Note that the tone threshold is around 90 dB attenuation which corresponds to roughly 35 dB SPL based on the calibration in Fig. 3.15 above, and this is as expected based on previous data, as seen in Fig. 2.15.

Overall, we found that ECochG responses could be recorded non-invasively with gold-foil tiptrodes from the ear canals of chinchillas. For anesthetized chinchillas, 300 polarity pairs appears to provide adequate responses.

3.3 Awake Restraint Setup

In order to record ECochG responses in awake chinchillas, an awake restraint set up was used. This set up was already established in the lab for other awake measurements, like middle ear muscle reflexes or distortion product otoacoustic emissions [24]. The restraint device consists of a PVC tube with a few rows of ventilation holes along the back to prevent overheating and also to help monitor breathing (upgraded from [25]), an example of which is shown in the appendix below. Once the chinchilla is in the tube and their head is positioned far enough out of the front of the tube, a neck collar attached on a hinge is closed over top and latched into place. The neck collar

provides enough room for the animal to still move around some, but keeps their head in an ideal position. In order to fix the head more firmly to minimize movement during data collection, the chinchilla is positioned such that a custom 3D-printed U-shaped nose holder is advanced over their nose and secured in place. The placement of the nose holder minimizes movement of the head while ensuring breathing is not obstructed and still allows for the proper placement of all necessary electrodes (in the ear canals and on the nose).

3.4 Results from Awake Recordings

The initial awake procedure was similar to the anesthetized procedures in that a range of attenuation levels was collected for both a click and 2 kHz tone stimulus. This initial procedure used 300 sets of each polarity with a filter width of 3 – 3000 Hz. The following awake procedures increased the sets of polarities to either 2,000 or 3,000 to help average out any remaining noise from ear canal measures; simple amplitude-based artifact rejection was used to remove large artifacts from animal movements made during the awake recording. The same calibrations from the final anesthetized procedure were used for all awake procedures. The other awake procedures collected responses to varying filter widths, varying attenuation levels, and varying frequencies of tone bursts. Two of the same chinchillas were tested multiple times in order to compare test/retest responses.

3.4.1 Stimulus and Response Timing

During one of the procedures, an acoustic click and an acoustic 2 kHz tone burst were recorded in the ear canal in order to document the onset of the stimulus. This was accomplished by making a small hole in the tiptrode for a probe microphone to fit through, and then the tiptrode and probe mic were placed in the ear canal. These acoustic responses were plotted with normal ECochG responses recorded from the tiptrodes that have been additionally notch filtered at 60 Hz. As shown in Figs. 3.22 and 3.23 below, it is clear that the onset of the stimulus and the onset of the response line up, indicating that the responses recorded are in fact acoustically-driven ECochG responses.

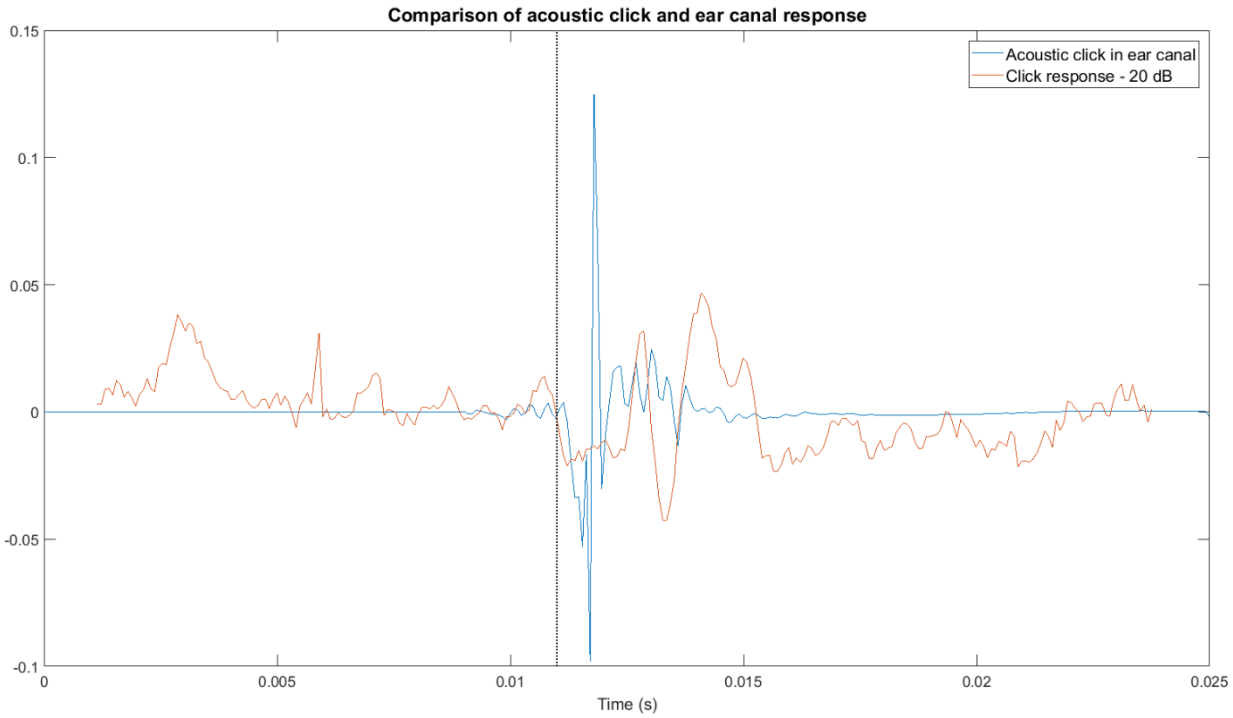

Figure 3.22 Comparison of acoustic click response in the ear canal of an awake chinchilla with click response recorded from a gold-foil tiptrode in the ear canal. The blue line is the acoustic click and the orange line is the response from the tiptrode. The response from the tiptrode has been notch filtered at 60 Hz. The vertical black line represents the approximate onset of the click stimulus.

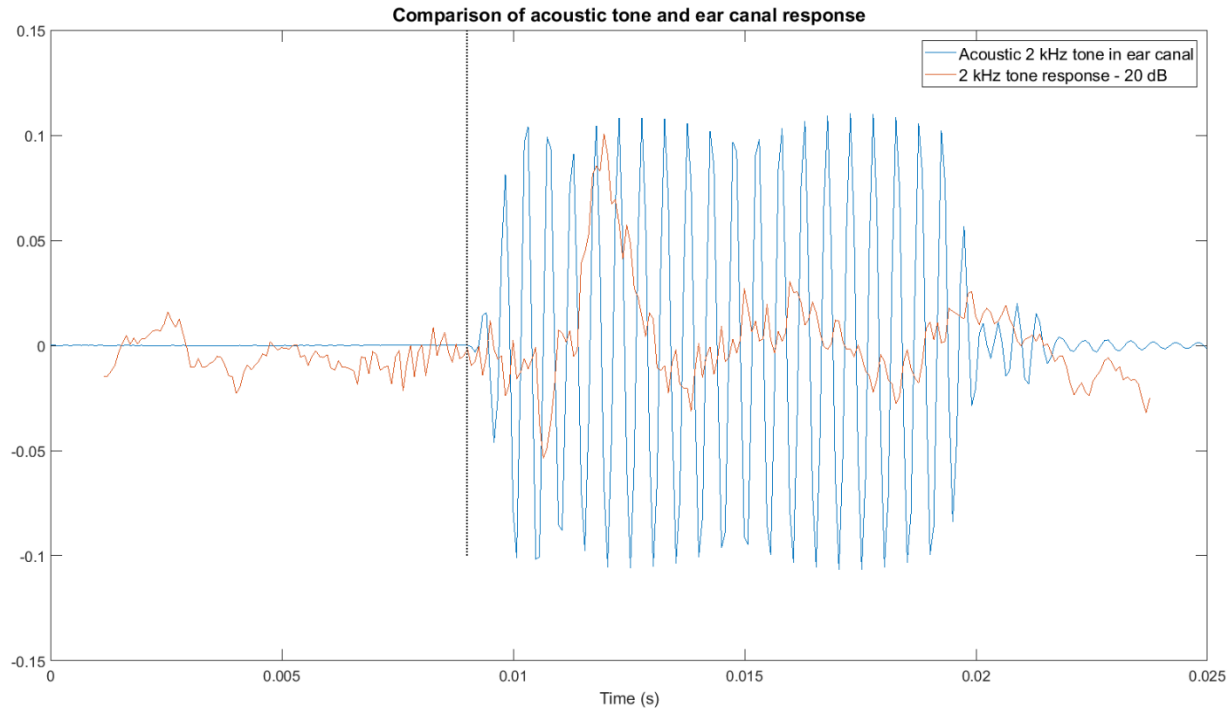

Figure 3.23 Comparison of acoustic 2 kHz tone burst in the ear canal with a tone response recorded from the gold-foil tiptrode in the ear canal of an awake chinchilla. The blue line is the acoustic tone burst and the orange line is the response from the tiptrode. The response from the tiptrode has been notch filtered at 60 Hz. The vertical black line represents the approximate onset of the tone stimulus.

3.4.2 Response Across Sound Level

As mentioned above, the first awake procedure collected responses to a click and a 2 kHz tone burst across a range of attenuation levels with 300 sets of each polarity. The comparisons of responses to the different levels are shown in Figs. 3.24 and 3.25 below. From the figures it is clear to see that the latency of the CAP response increases as attenuation level increases, or as sound level decreases, for both the clicks and tones. As a reminder, the approximate sound level for a click at 40 dB attenuation is about 65 dB SPLpeak, and the approximate sound level for a 2 kHz tone is about 85 dB SPL based on the calibrations described above (Figs. 3.15-3.16). This increase in latency with decreasing sound level suggests that the responses are cochlear, consistent with them being ECochG responses. It is also clear from the figures that the overall response amplitude decreases with decreasing sound level as expected, and disappears below threshold.

One important thing to note about these initial comparison figures is that they have been notch filtered at 60 Hz to remove lingering power line noise. Though this filtering helps remove

noise and variability, especially before and after the ECochG response as shown above in Figs. 3.11 and 3.12, the filtering is not perfect. For these recordings, we used a relatively low high-pass cutoff value in order to optimize the SP measures, but a tradeoff is required to eliminate the baseline variability.

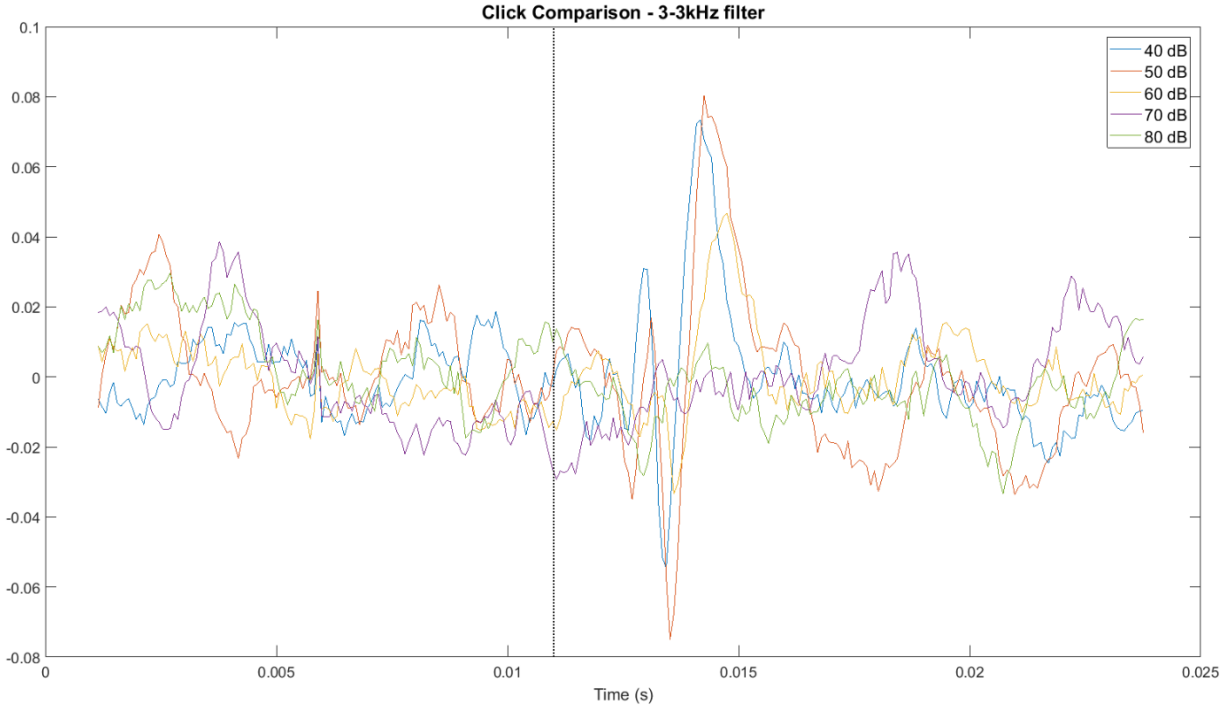

Figure 3.24 Comparison of click responses from the initial awake procedure for a range of attenuation levels below and above threshold showing the shift in latency. The vertical dotted black line indicates the approximate onset of the click stimulus. The line colors correspond to the different attenuation levels. Responses have been notch filtered.

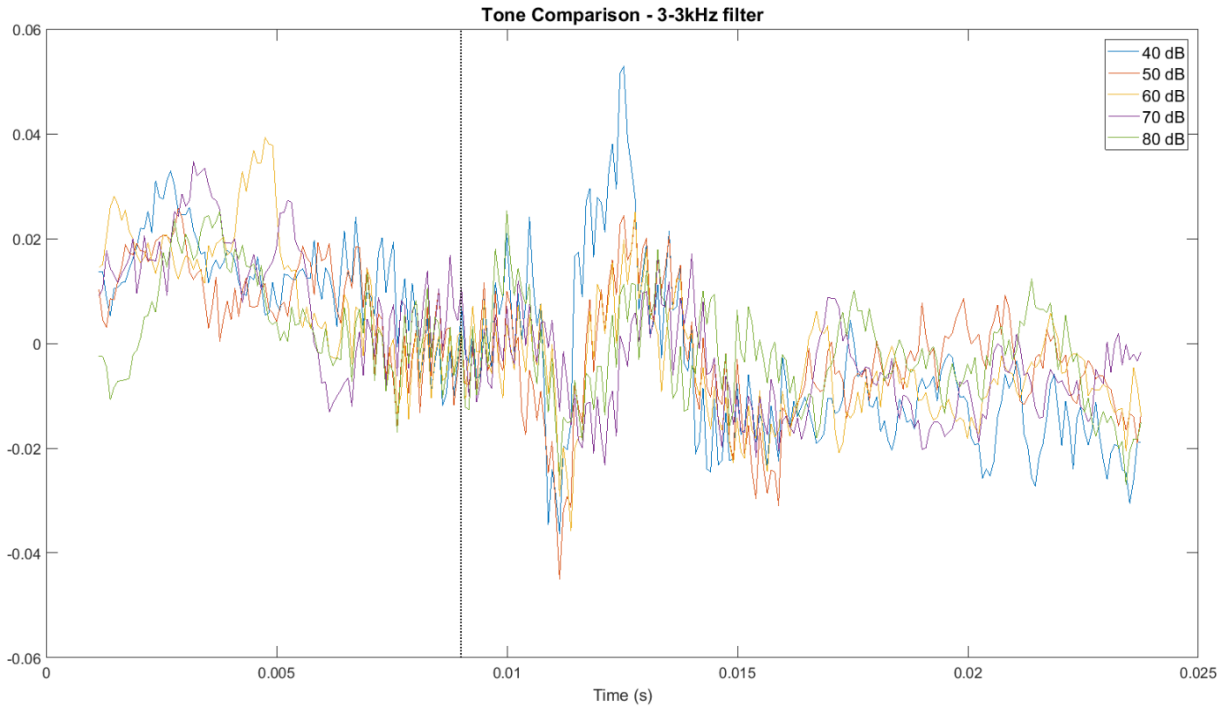

Figure 3.25 Comparison of 2kHz tone responses from the initial awake procedure for a range of attenuation levels above and below threshold showing the shift in latency. Responses to alternating polarities were averaged. The vertical dotted black line represents the approximate onset of the tone stimulus. The line colors correspond to the different attenuation levels. Responses have been notch filtered.

After the initial awake experiment, the following awake experiments increased the number of sets of polarities to either 2,000 or 3,000 in order to help average out some of the noise and obtain cleaner signals (at the expense of the number of stimulus conditions that could be recorded). During two of these awake experiments, responses to clicks were collected at 30 dB and then 50 dB attenuation from two different animals in order to more clearly see any shift in latency with changing attenuation level. The first example of this is shown in Fig. 3.26 below where there is a clear shift in latency between the two responses, with latency increasing as attenuation level increases (i.e., sound level decreases) as expected from cochlear responses. The second example of this is shown in Fig. 3.27 below where there is again a clear shift in latency between the responses with latency increasing as attenuation level increases. During one of the awake experiments, responses to a 2 kHz tone were collected at 25 dB and 50 dB, as shown in Fig. 3.28 below. As with the click responses, there is a clear increase in latency as attenuation level increases. These shifts in latency are consistent with these being ECochG responses.

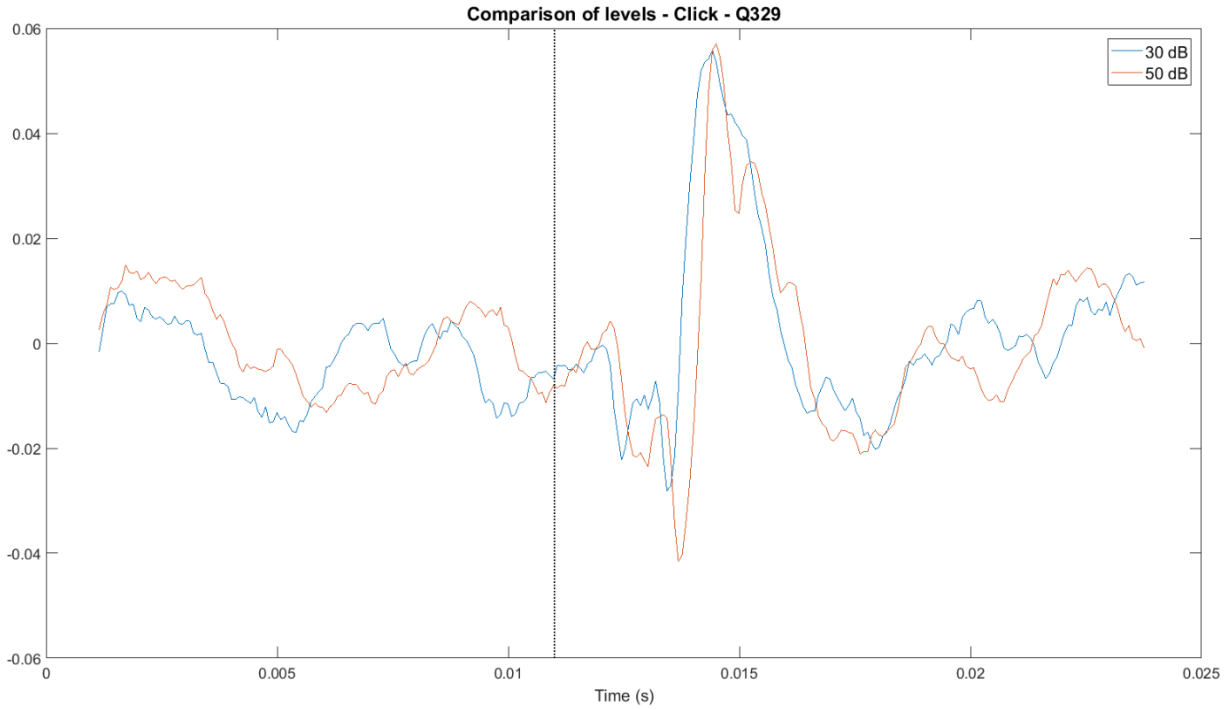

Figure 3.26 Comparison of click responses from two different attenuation levels from chinchilla number Q329 during the third awake procedure with a clear shift in latency as sound level decreases. There were 3,000 sets of each polarity presented. The vertical black line represents the approximate onset of the click stimulus. Responses have been notch filtered.

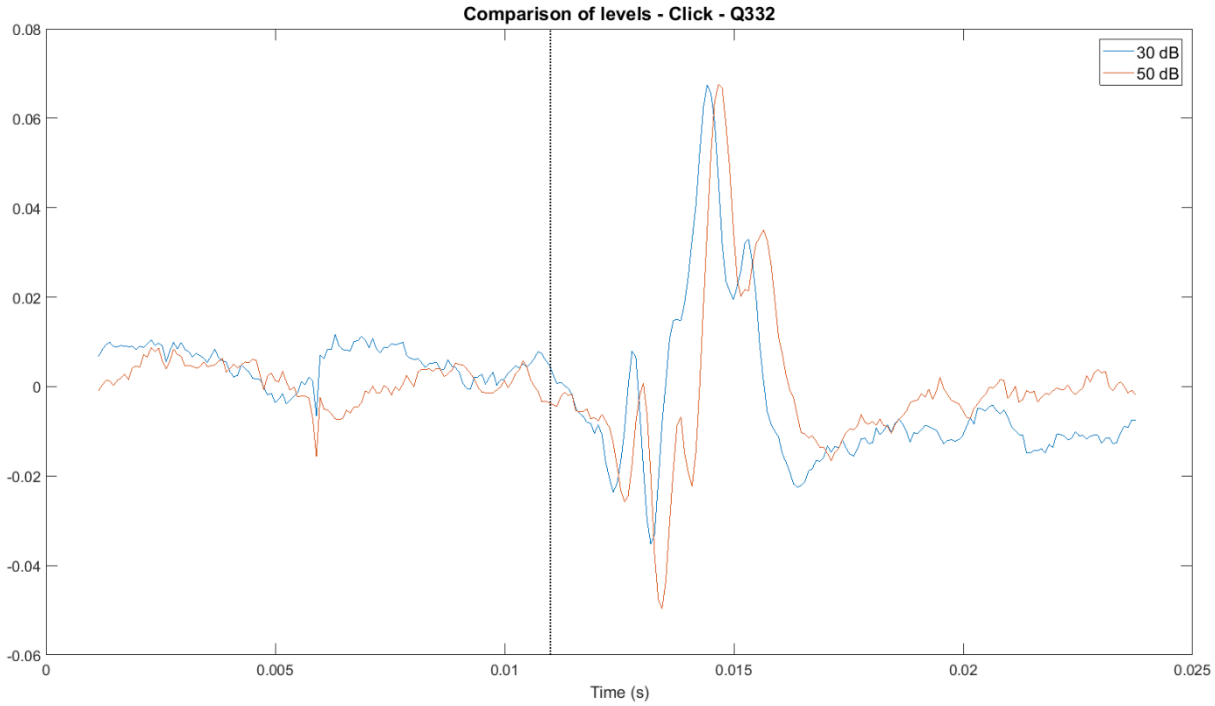

Figure 3.27 Comparison of click responses from two different attenuation levels from chinchilla number Q332 during the final awake procedure with a clear shift in latency as sound level decreases. There were 2,000 sets of each polarity presented. The vertical black line represents the approximate onset of the click stimulus. Responses have been notch filtered.

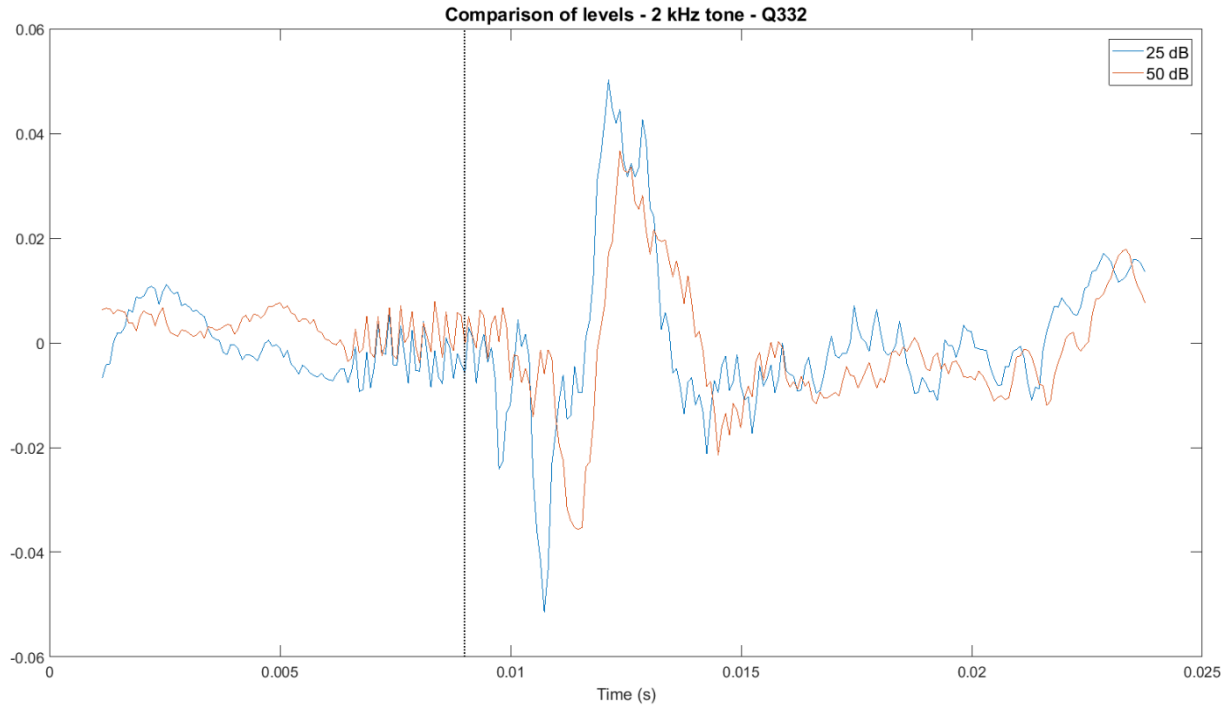

Figure 3.28 Comparison of 2kHz tone responses at two different attenuation levels from Q332 during the last awake procedure showing a clear shift in latency with decreasing sound level. There were 2,000 sets of each polarity presented. The vertical black line represents the approximate onset of the tone stimulus. Responses have been notch filtered.

During the final awake experiment, responses to clicks were collected for attenuation levels ranging from 20 dB to 70 dB in 10 dB steps in order to better characterize how latency and amplitude change with changing sound level. The initial responses to these attenuation levels are shown in Fig. 3.29 below where it can be seen that latency and amplitude do change with attenuation level. In order to actually quantify this change though, the initial metrics described in Chapter 2 above were calculated on these responses in order to determine N1 and its latency and amplitude, an example of which is shown in Fig. 3.30 below. As a reminder, N1 was chosen as the largest negative peak, which for this awake data was always the second negative peak. It should be noted that the first peak in the response may be an onset SP, but additional experiments would be required to confirm whether it derives from hair cells or if it is neural (e.g., using tetrodotoxin to eliminate neural responses [26]). Once N1 was identified for all attenuation levels, the latency and amplitude were plotted versus level as shown in Figs. 3.31 and 3.32 below. From these figures it is clear to see that latency increases with increasing attenuation level (i.e., decreasing sound

level) and amplitude decreases with increasing attenuation level. These changes in latency and amplitude are consistent with these being ECochG responses.

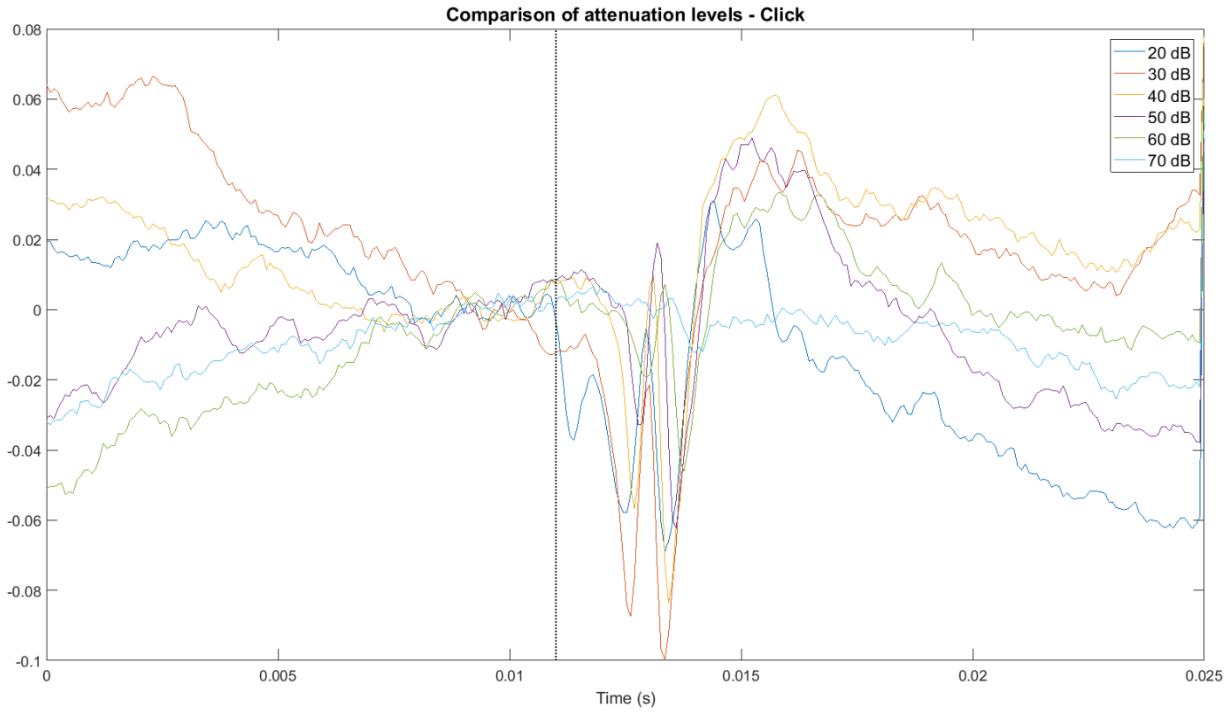

Figure 3.29 Comparison of click responses at varying attenuation levels from Q393 during the final awake procedure. There were 2,000 sets of each polarity presented. The vertical black line represents the approximate onset of the click stimulus. The line colors correspond to the different attenuation levels. Responses have been individually baseline corrected where the baseline window is from 8-11 ms.

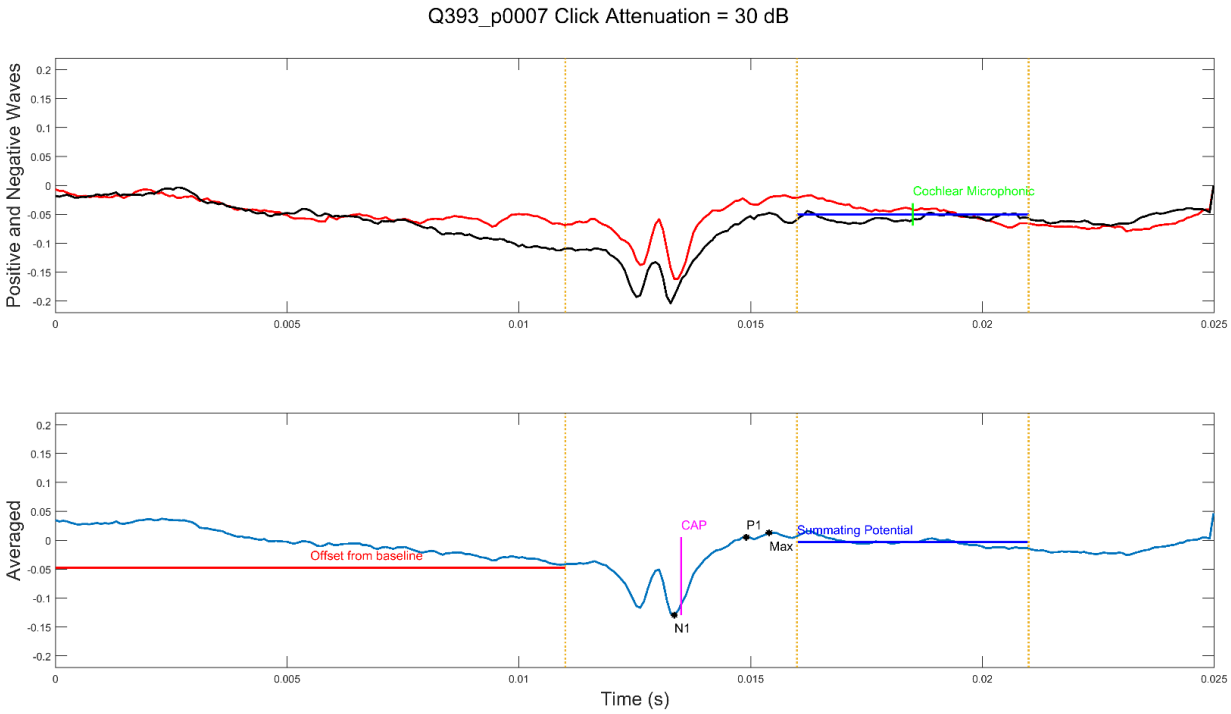

Figure 3.30 Example of the calculated initial metrics on one of the awake click recordings. The metrics were calculated exactly as described in Chapter 2 above. These metrics were mainly used to determine N1 and its latency and amplitude in order to plot latency and amplitude versus level.

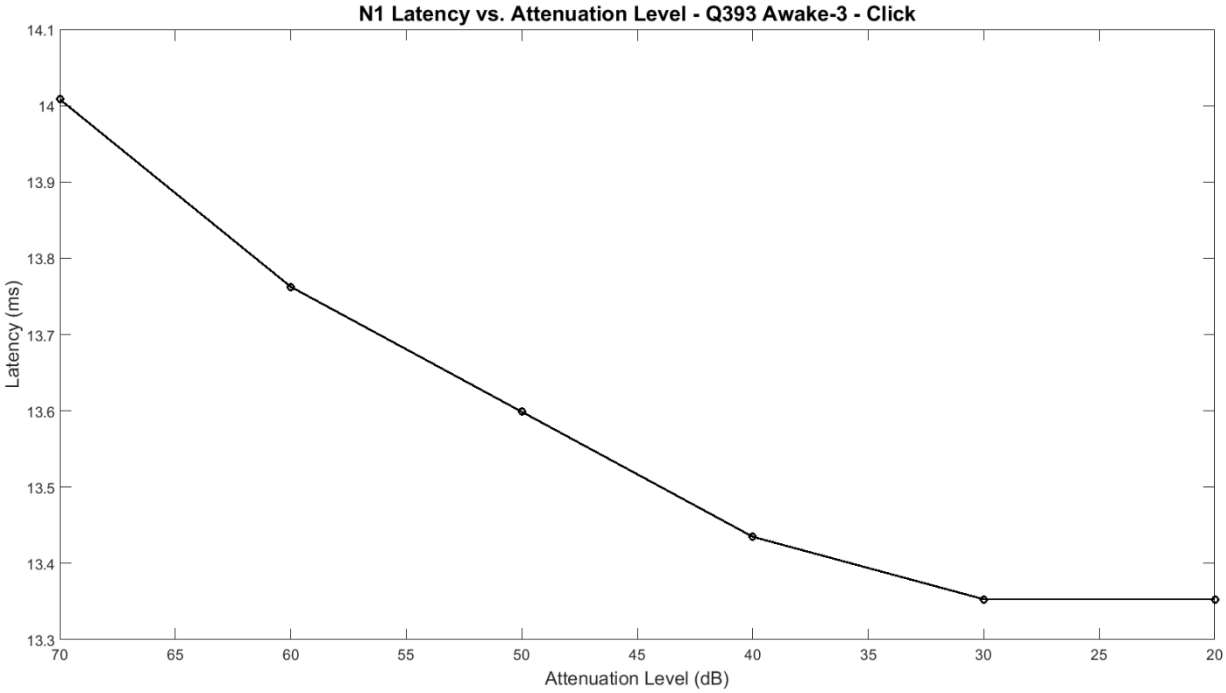

Figure 3.31 Graph of the latency of calculated N1 versus attenuation level for click responses from chinchilla Q393 during the final awake procedure showing a clear increase in latency with decreasing sound level. The latency has been converted from seconds to milliseconds. Note that increasing attenuation level is equivalent to decreasing sound level. Based on a calibration in one ear during the final anesthetized procedure (Fig. 3.16), a value of 20 dB attenuation roughly corresponds to 85 dB SPLpeak for a click response.

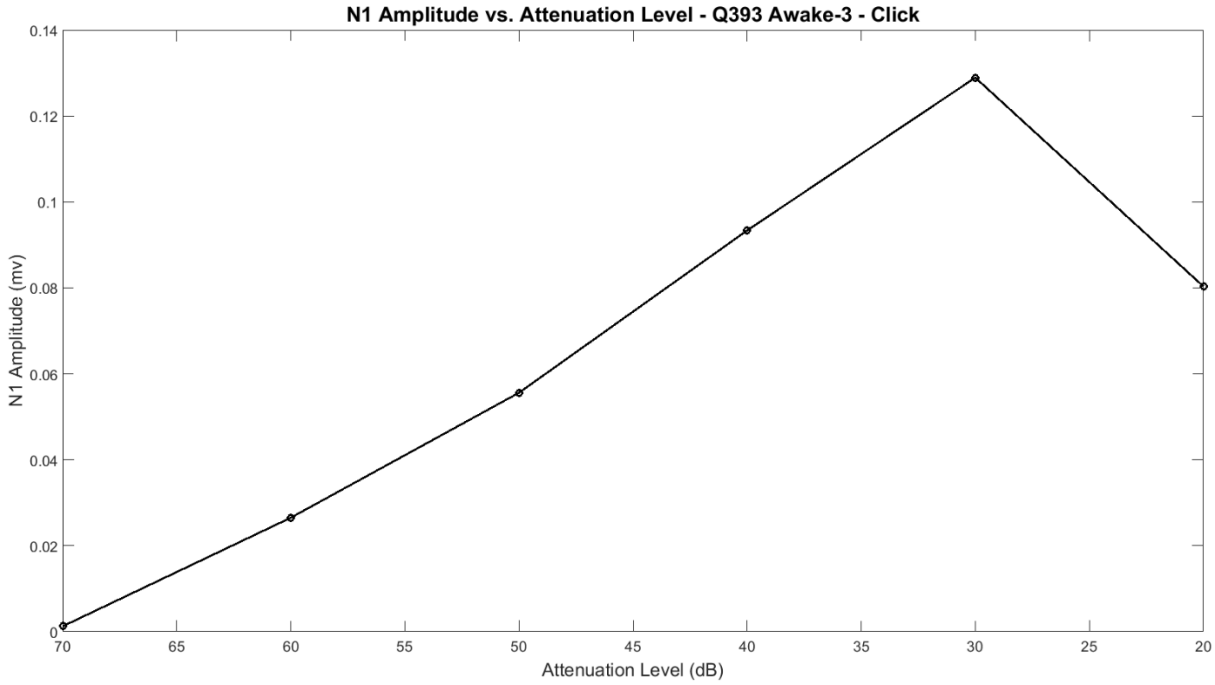

Figure 3.32 Graph of the absolute value of the N1 amplitude versus level for click responses from chinchilla Q393 during the final awake procedure showing a clear trend of increasing amplitude with increasing sound level. The N1 amplitude was calculated as the absolute value of the difference between baseline (which is corrected to be zero) and the N1 peak (same data as in Fig. 3.31). Note that decreasing attenuation level is equivalent to increasing sound level. The highest level shows a reduced peak, as seen in previous figures, most likely due to some form of saturation.

3.4.3 Responses Across Tone Frequency

For two chinchillas, a complete range of tone frequency responses were collected at the same attenuation level. For one chinchilla, the tones were collected at a sound level well above threshold, shown in Fig. 3.33 below, while for the other the tones were presented at a sound level closer to threshold, shown in Fig. 3.36 below. In order to compare latencies across the different tone frequencies, the initial metrics were calculated on the awake data in order to determine N1 and its latency, examples of which are shown in Figs. 3.34 and 3.37 below. These latencies were then plotted versus frequency, as shown in Fig. 3.35 below, and for the first chinchilla with responses well above threshold, there is a clear decrease in latency as frequency increases from 500 Hz to 8,000 Hz. It should be noted that when tones are presented at sound levels much greater than threshold, cochlear spread of excitation occurs, meaning that lower frequencies activate more basal regions of the cochlea and the tonotopic latency/frequency tradeoff becomes less prominent. For the tones presented at a sound level closer to threshold, there is a consistent decrease in latency

as frequency increases, shown in Fig. 3.38 below, which is as expected based on cochlear tonotopicity.

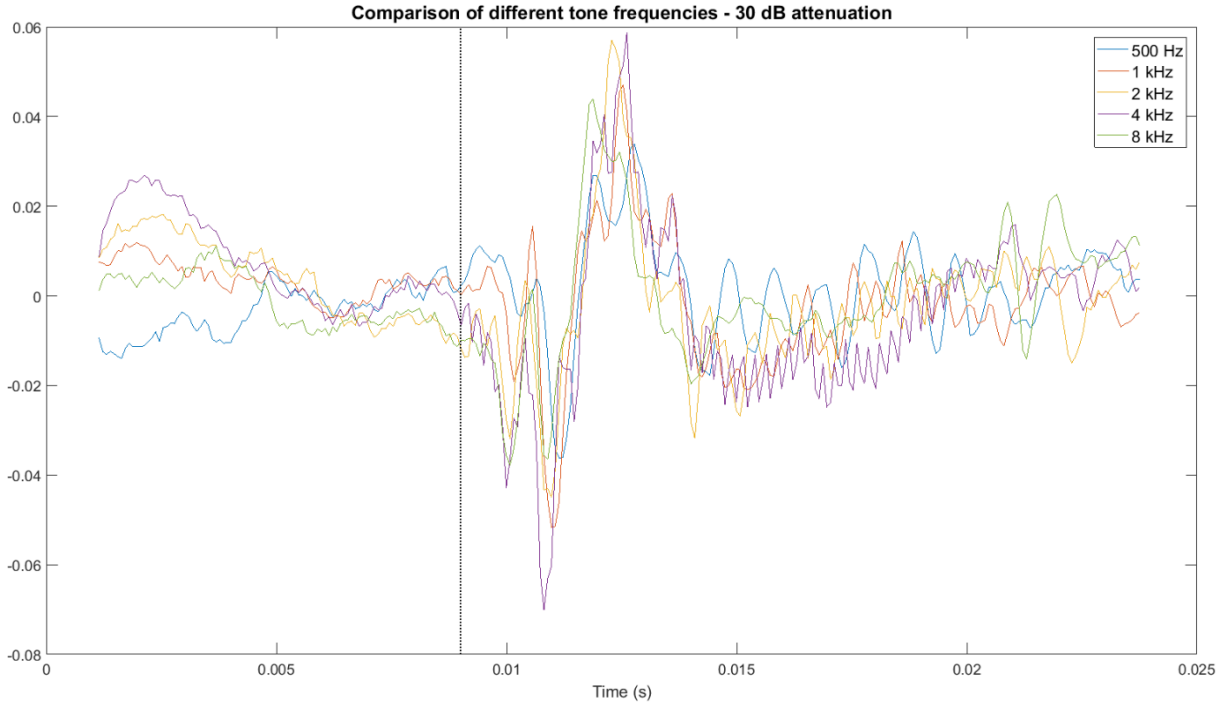

Figure 3.33 Comparison of the awake ECochG responses to different tone burst frequencies at the same attenuation level (high SPL) showing a latency shift for the lower frequencies. The vertical dotted black line represents the approximate onset of the tone burst stimuli. The line colors correspond to the different frequencies. Responses have been notch filtered.

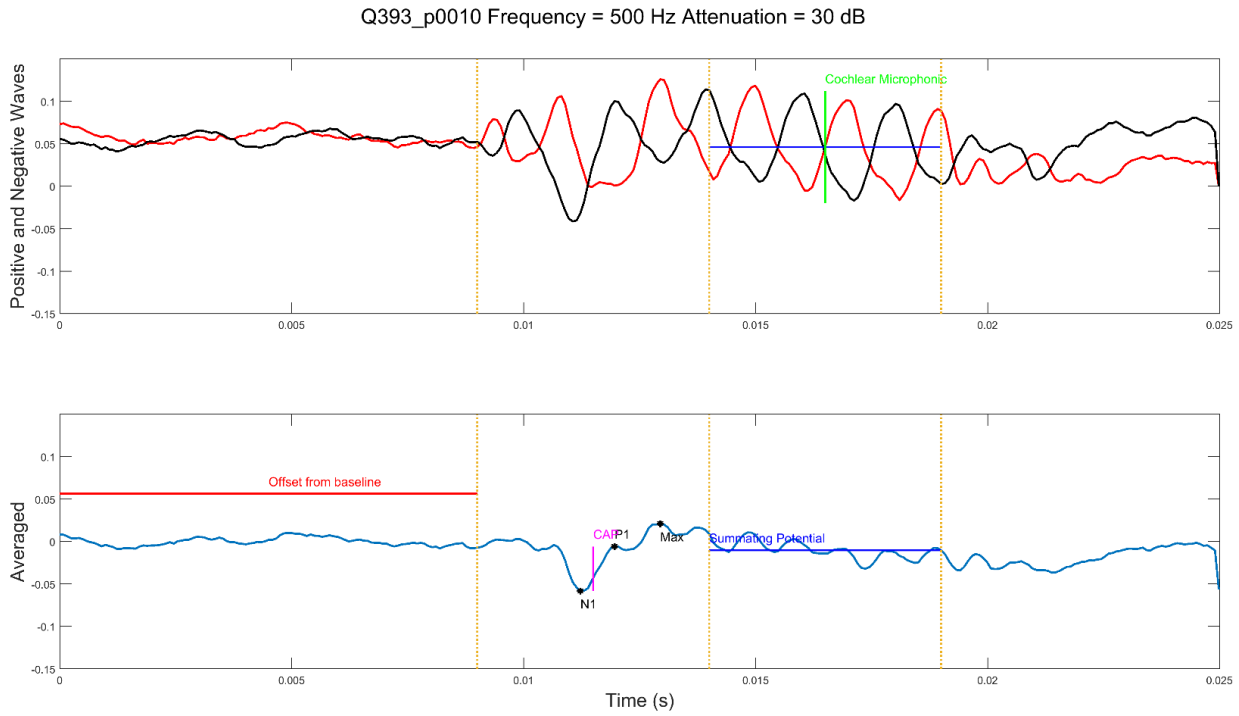

Figure 3.34 Example of the calculated initial metrics on one of the awake recordings. The metrics were calculated exactly as described in Chapter 2 above, and it should be noted that with the new recording setup for the awake procedures, the calculation of the metrics may need to be adjusted, especially where and how the SP and CM are calculated (not computed here). Here, we only determined N1 and its latency in order to plot latency versus level.

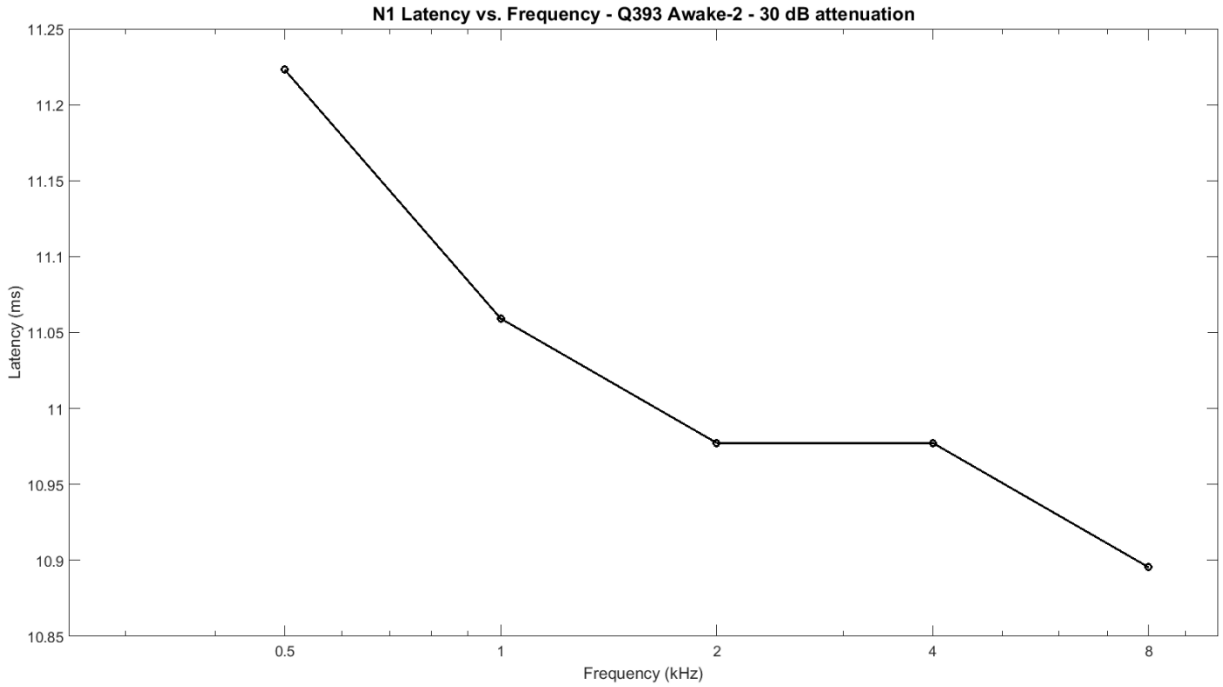

Figure 3.35 Graph of N1 response latency versus frequency for different tone burst responses at the same attenuation level of 30 dB (high SPL). Responses are from Q393 during the second to last awake procedure. Even at a relatively loud attenuation level, there is a clear trend of decreasing latency with increasing frequency.

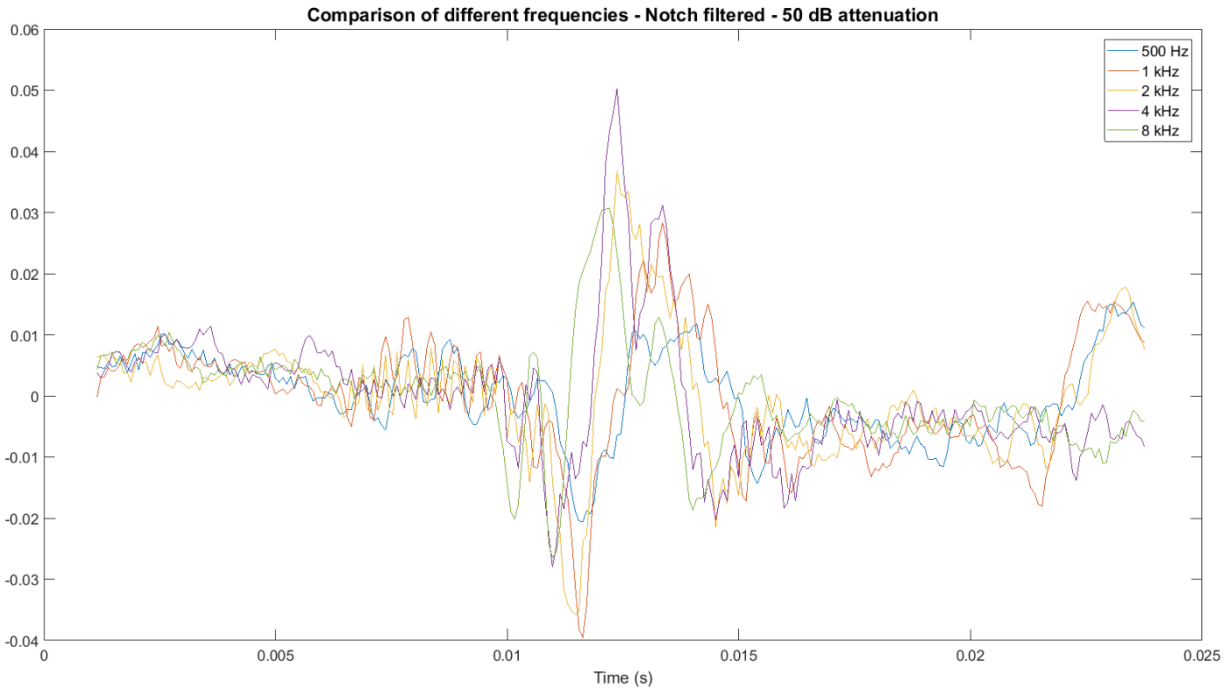

Figure 3.36 Comparison of the awake ECochG responses to different tone burst frequencies at the same attenuation level of 50 dB which is closer to threshold. Note that these responses are from a different chinchilla than the other set of frequency responses shown above. The line colors correspond to the different frequencies. Responses have been notch filtered.

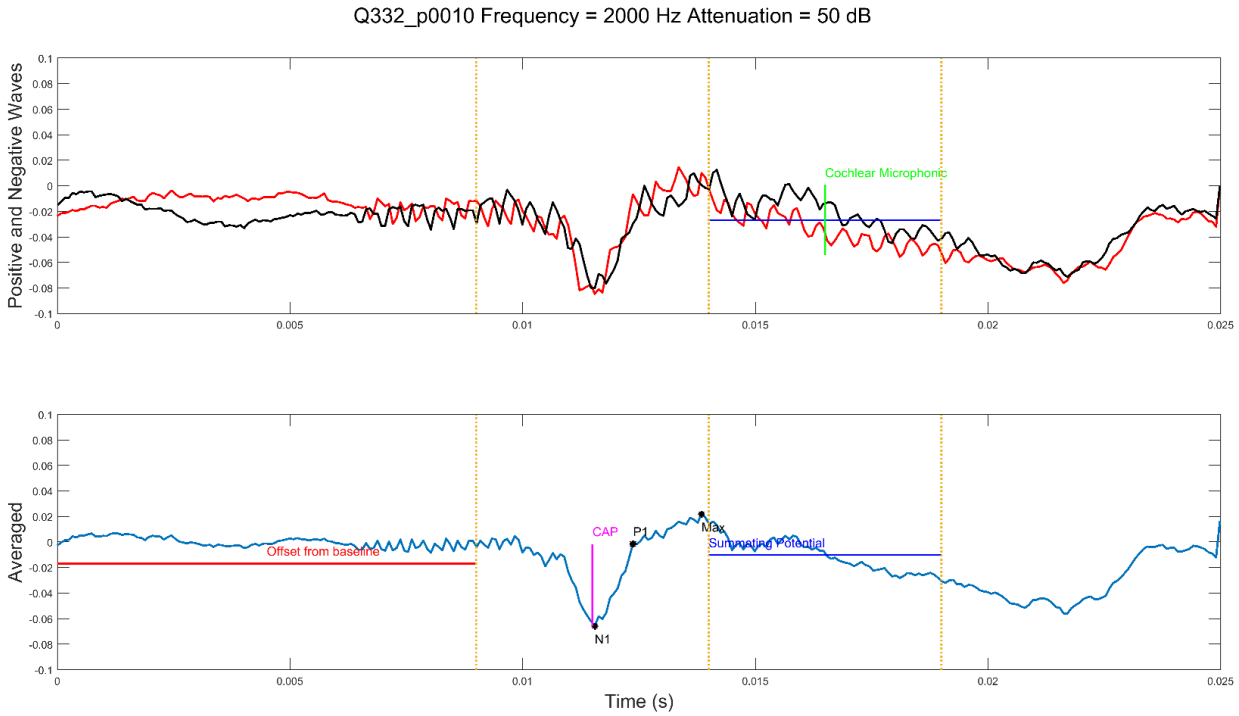

Figure 3.37 Example of the calculated initial metrics on one of the awake recordings. The metrics were calculated exactly as described in Chapter 2 above, and it should be noted that with the new recording setup for the awake procedures, the calculation of the SP and CM metrics may need to be adjusted (not computed here). We only determined N1 and its latency here in order to plot latency versus level.

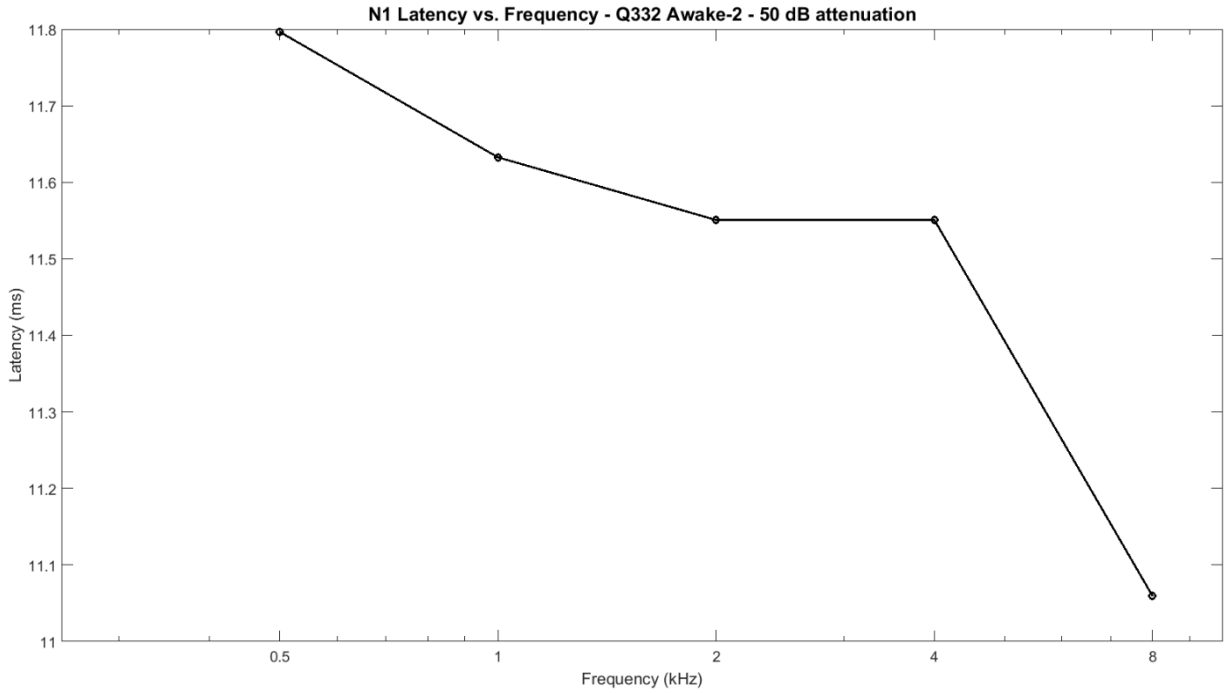

Figure 3.38 Graph of N1 response latency versus frequency for different tone burst responses at the same attenuation level of 50 dB (~20-30 dB above threshold). Responses are from chinchilla Q332 during the final awake procedure. Latency has been converted from seconds to milliseconds. There is a stronger trend of decreasing latency as frequency increases at this lower SPL compared to the higher SPL responses shown in Fig. 3.35.

3.4.4 Test/Retest Responses

There were two chinchillas whose responses were collected across different weeks in order to compare test/retest of responses. For one chinchilla, there were two conditions that were collected two different times across the different weeks. This first condition was a click stimulus at 40 dB attenuation level with 0.3 – 3000 Hz filter width, and the second condition was a click stimulus at 40 dB attenuation level but with 30 – 3000 Hz filter width. The comparisons of the responses, which have been notch filtered, across time are shown in Figs. 3.39 and 3.40 below. Though there were some differences in baseline noise levels, the ECochG responses were very similar, showing that the responses are real and repeatable across multiple experiments on different days.

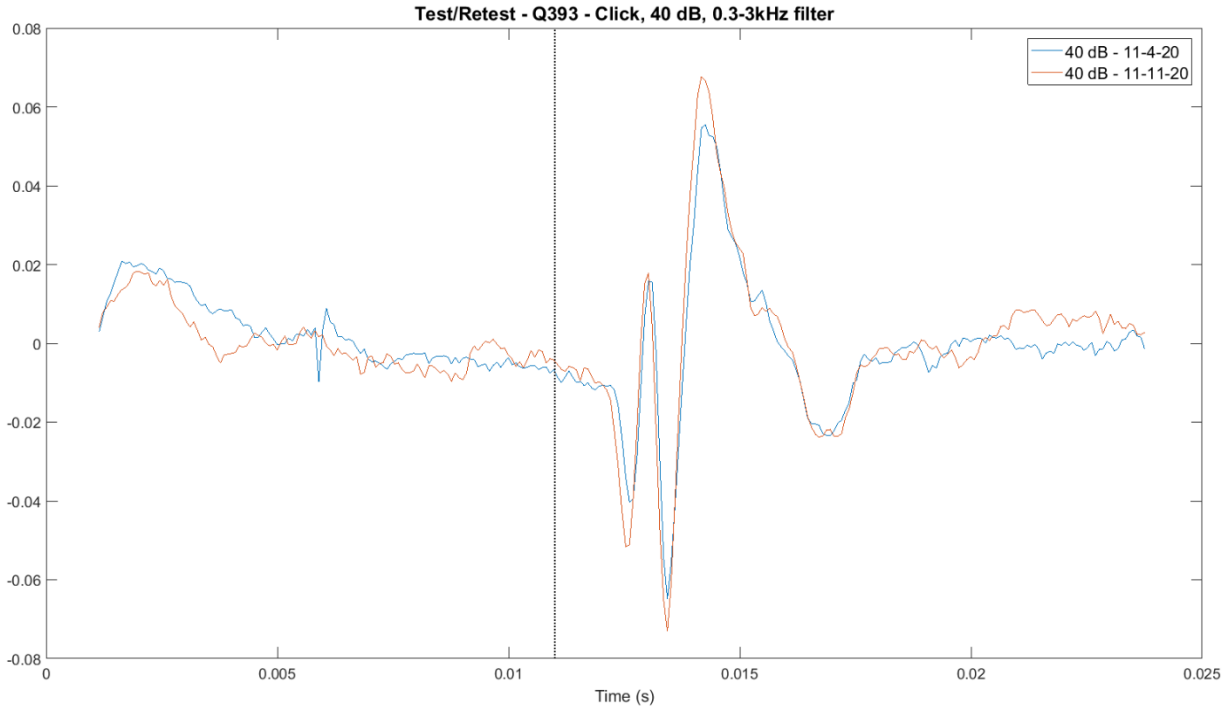

Figure 3.39 Comparison of test/re-test responses to a click stimulus at 40 dB attenuation with a filter width of 0.3-3000 Hz in the same chinchilla a week apart. The blue line is the first response and the orange line is the response to the same stimulus a week later. The vertical black line represents the approximate onset of the click stimulus. Responses have been notch filtered.

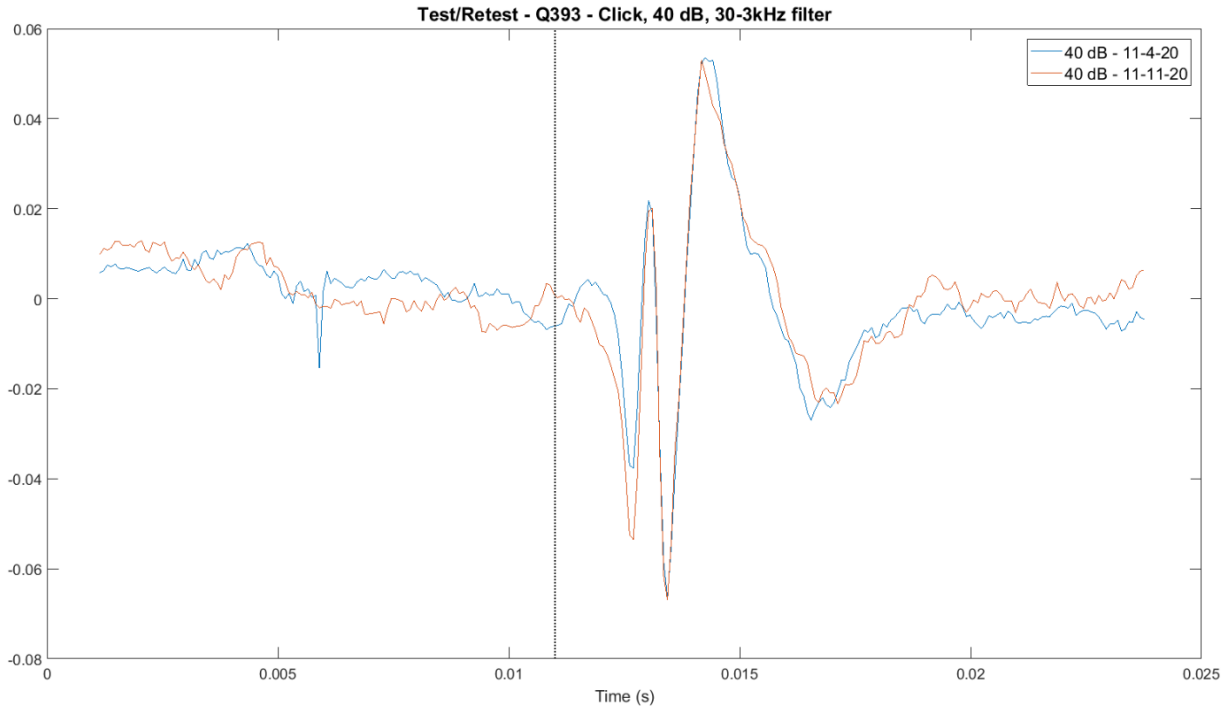

Figure 3.40 Comparison of test/re-test responses to a click stimulus at 40 dB attenuation with a filter width of 30-3000 Hz in the same chinchilla a couple of weeks apart. The blue line is the first response and the orange line is the response to the same stimulus a week later. The vertical black line represents the approximate onset of the click stimulus. Responses have been notch filtered.

For the second chinchilla, there were again two conditions that were collected at two different times across different weeks. The first condition was a click stimulus at 30 dB attenuation level with a filter width of 0.3 – 3000 Hz, and the second condition was a 2 kHz tone burst at 25 dB attenuation level with a filter width of 0.3 – 3000 Hz. The filtered responses are shown in Figs. 3.41 and 3.42 below. As with the first chinchilla, though there were differences in baseline noise levels, the ECochG responses are quite similar.

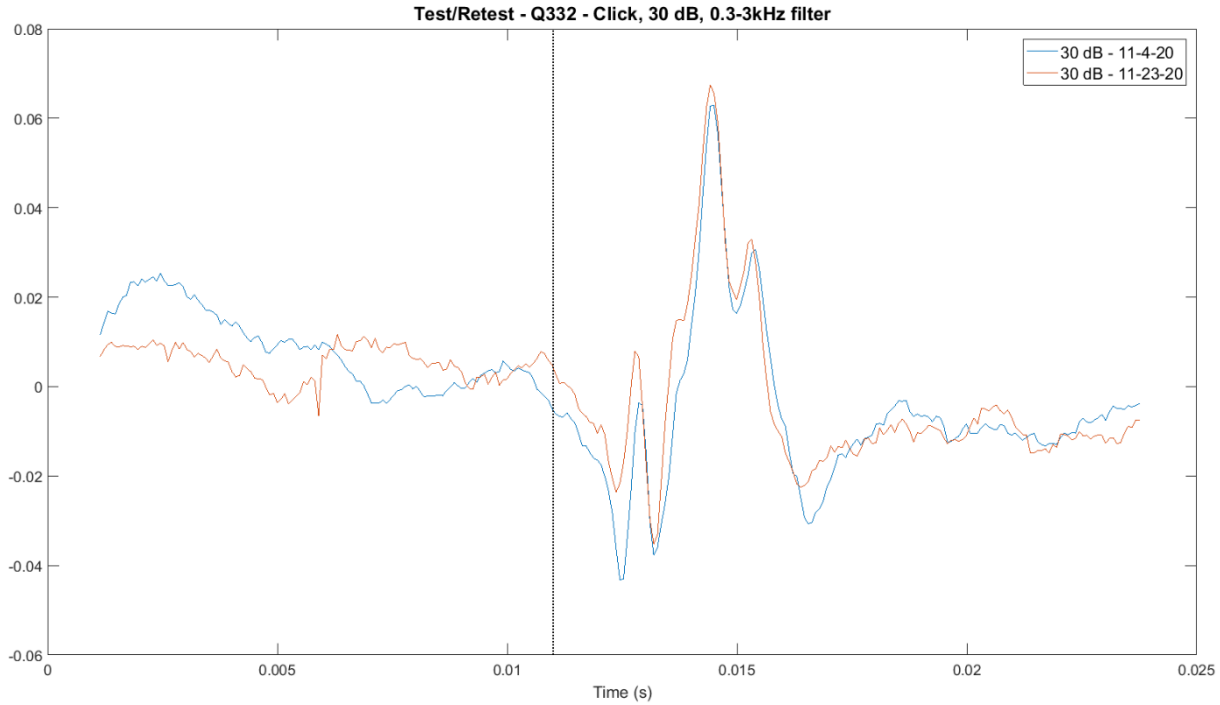

Figure 3.41 Comparison of test/re-test responses to a click stimulus at 30 dB with a filter width of 0.3-3000 Hz in the same chinchilla a couple of weeks apart. The blue line is the first response and the orange line is the response to the same stimulus two weeks later. The vertical black line represents the approximate onset of the click stimulus. Responses have been notch filtered.

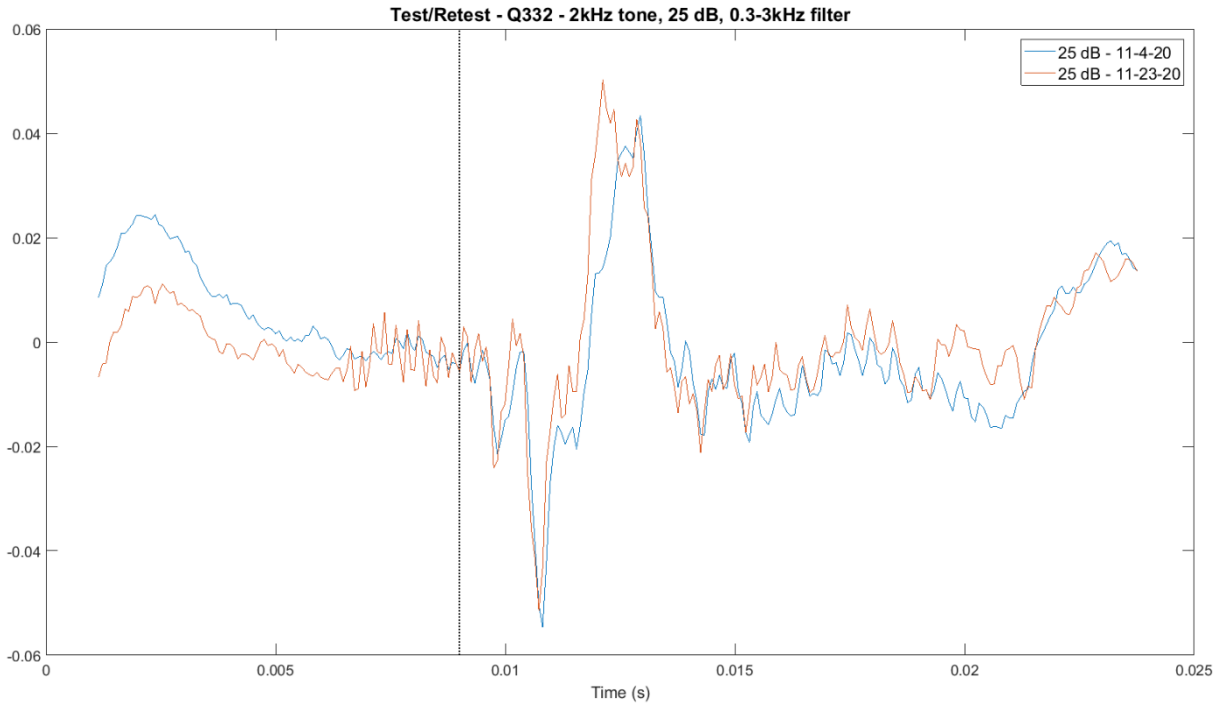

Figure 3.42 Comparison of test/re-test responses to a 2 kHz tone stimulus at 25 dB with a filter width of 0.3-3000 Hz in the same chinchilla a couple of weeks apart. The blue line is the first response and the orange line is the response to the same stimulus two weeks later. The vertical black line represents the approximate onset of the tone stimulus. Responses have been notch filtered.

4. ADDITIONAL ELECTROCOCHLEOGRAPHY MEASURES

4.1 Advanced Complementary Metrics from Harris et al. 2018

In their paper "Complimentary metrics of human auditory nerve function derived from compound action potentials", Harris et al. developed several metrics for an ECochG response to be used individually or in combination to help characterize AN function and fiber loss in humans non-invasively [27]. The five metrics they used were peak amplitude, peak latency, onset latency, half width, and area of the wave. They elicited responses with clicks that were 0.1 ms in duration with alternating polarity and presented at 11.1/s at sound levels from 70 – 100 dB pSPL in 10 dB steps [27]. In order to compare our results with theirs, we applied these same metrics just to the click responses from the data mentioned in Chapter 2. One significant difference between the Harris results and our data, aside from the difference in species (i.e., humans vs. chinchillas), is that Harris tested at suprathreshold sound levels while our data included some levels below threshold as well as many above threshold, though most of our highest levels used were still at or below the lowest sound level Harris used. In order to help with the calculation of the metrics as well as to be as close in sound level to Harris as possible, the metrics were only applied to responses from sound levels at least one 5 dB step above the CAP threshold and above.

For the peak amplitude and latency, the values calculated in section 2.3 above for the CAP were used with N1 as the peak and the amplitude equal to the value of the peak since the averaged waveform was corrected so the baseline was zero. The onset of N1 was calculated as the point back in time from the peak latency where the wave reached 10% of the peak amplitude. The half-width of the wave was calculated as the difference between the onset latency and the peak latency. To calculate the area, the 10% fraction of the peak was determined forward in time (i.e., after N1) as the offset of the wave, and the onset and offset points were used to perform numerical integration of the wave using the trapz function in MATLAB. The calculations for the onset, offset, and area were modeled after functions used in the *ERPLab* toolbox [28] which Harris et al. used in their calculations [27]. These metrics were then plotted on the response waveform, as shown in Fig. 4.1 below.

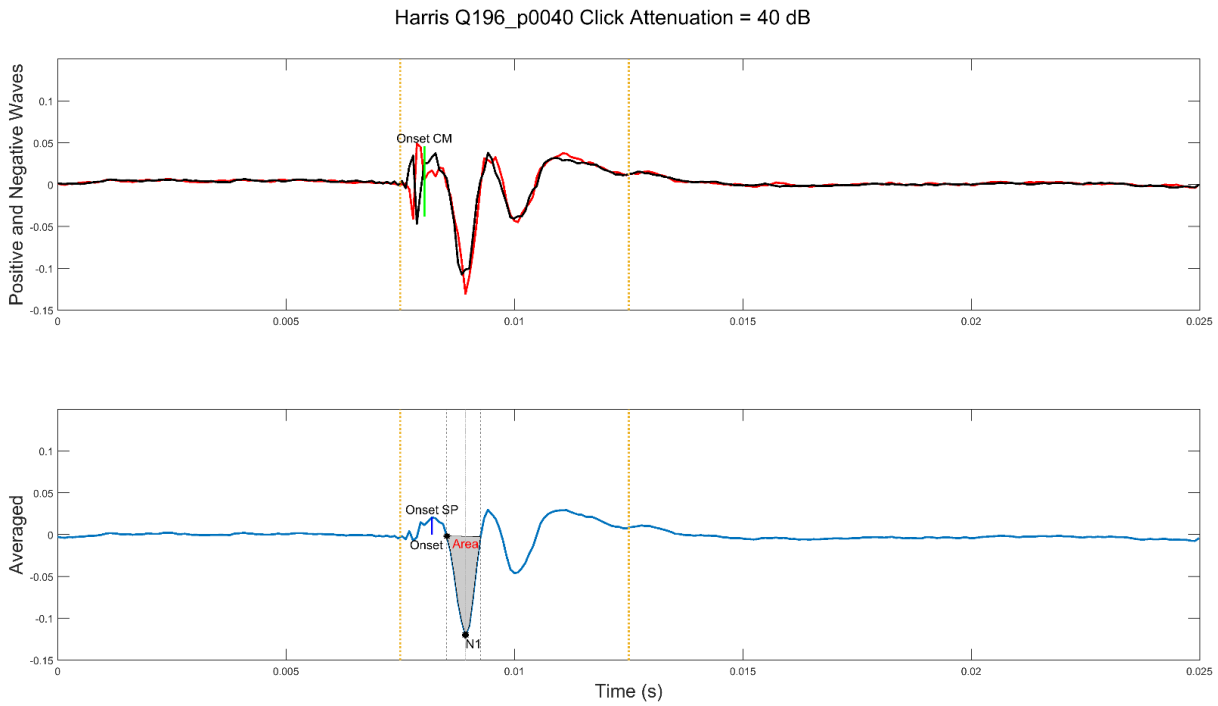

Figure 4.1 Example of the Harris and onset metrics plotted on the response waveforms. The top subplot shows the positive and negative polarity averages in mv from which the onset CM was calculated. The bottom subplot shows the averaged response in mv from which all the other metrics were calculated. The gray vertical dotted lines represent the onset and offset of the N1 wave. The filled in gray area represents the calculated area of the curve between the onset and offset.

After the metrics were calculated for all of the sound levels that met the aforementioned criteria, they were plotted versus level, as shown in Fig. 4.2 below. Once all of the individual metrics were plotted versus level, the metrics were averaged from the different data files for the same chinchilla, and then averaged across chinchillas with the same hearing type. As with the initial metrics, if there was only one data point at a given level, it was not included in the average. Finally, the three different hearing types were plotted versus level for the metrics in order to identify any trends in the metrics. Examples of these plots are shown in Figs. 4.3-4.5 below. Overall, the Harris metrics demonstrate trends in our chinchilla data that are broadly consistent with their human results (e.g., peak amplitude and area increased with increasing sound level while peak latency decreased with increasing sound level, as seen in Harris et al.'s Fig. 5). This demonstrates that these more detailed metrics can be computed from chinchilla data, and may be useful diagnostics when a larger chinchilla data set becomes available (e.g., with cochlear synaptopathy).

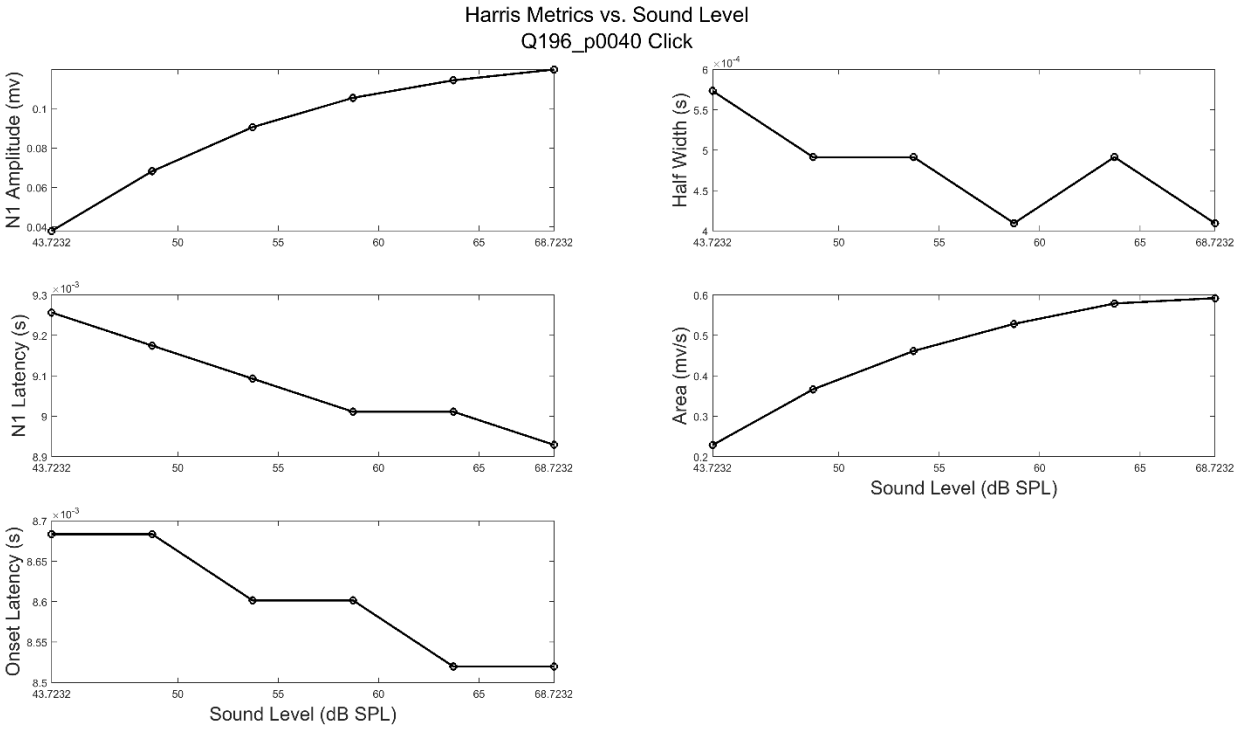

Figure 4.2 Example of the different Harris metrics plotted versus sound level for click responses from a single data file in a NH chinchilla (Q196).

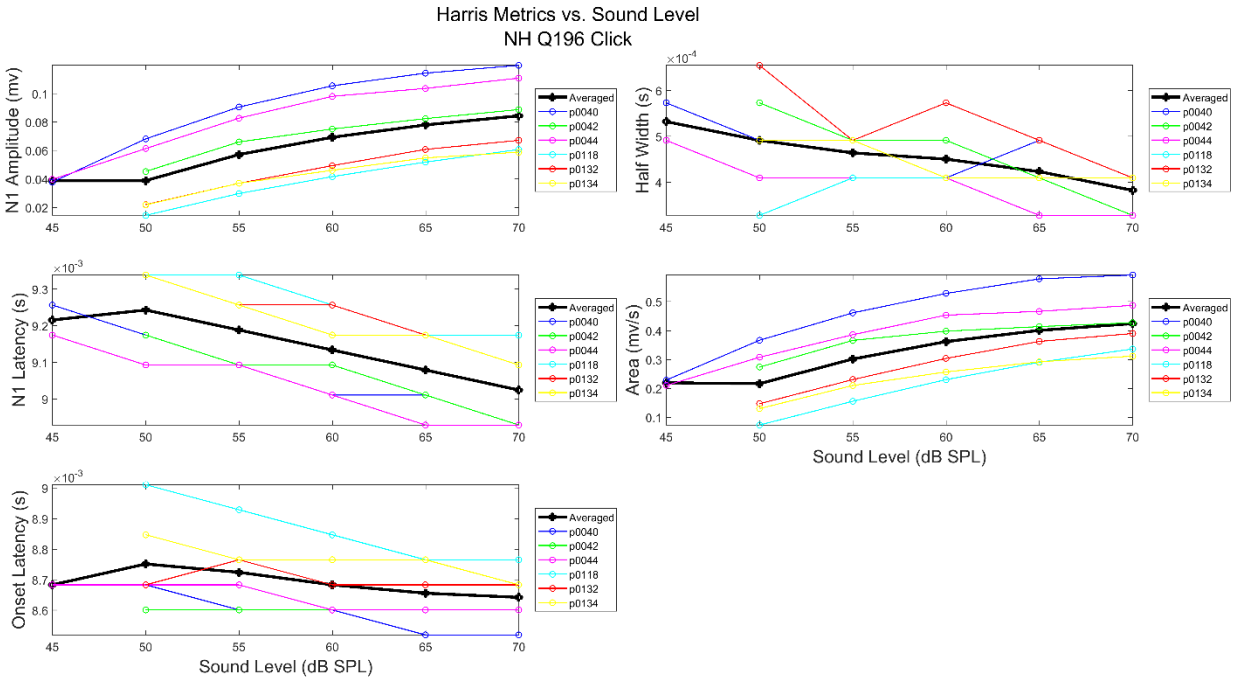

Figure 4.3 Example of the different Harris metrics plotted versus sound level averaged across click data files for one chinchilla. The thicker black lines are the averaged metrics and the line colors correspond to the different data files.

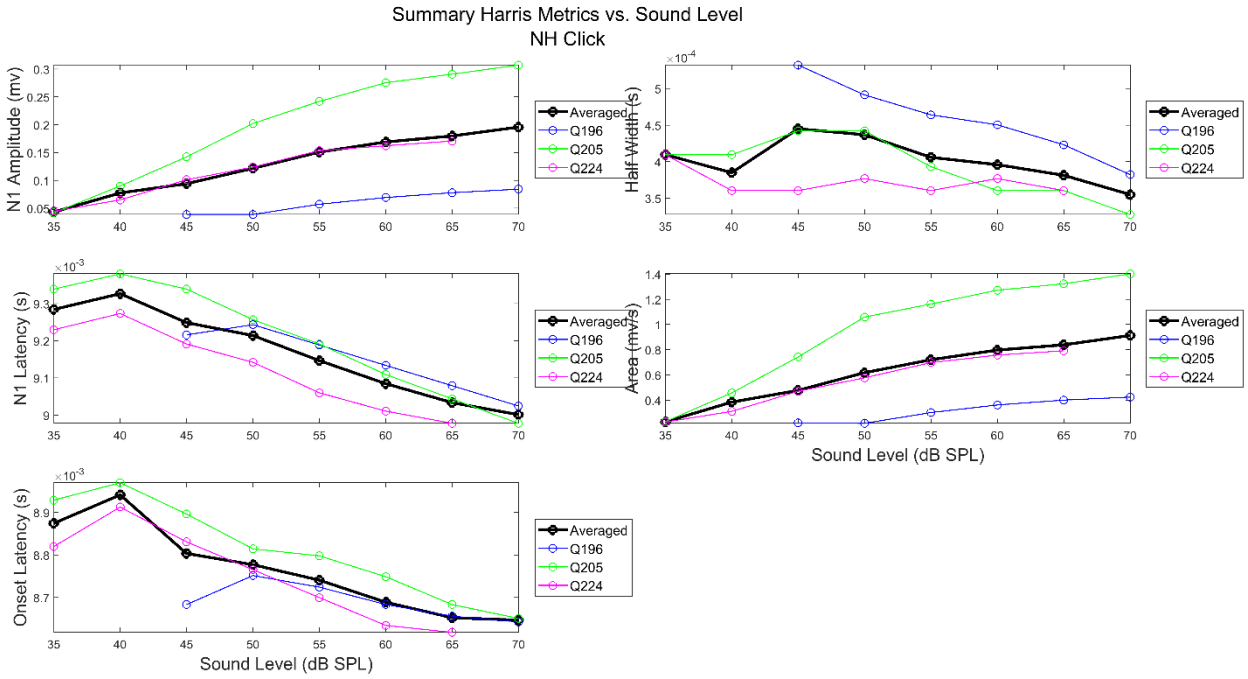

Figure 4.4 Example of the different Harris metrics averaged across chinchillas with the same hearing type (NH). The thicker black lines are the averaged metrics and the line colors correspond to the different chinchillas with the specified hearing type.

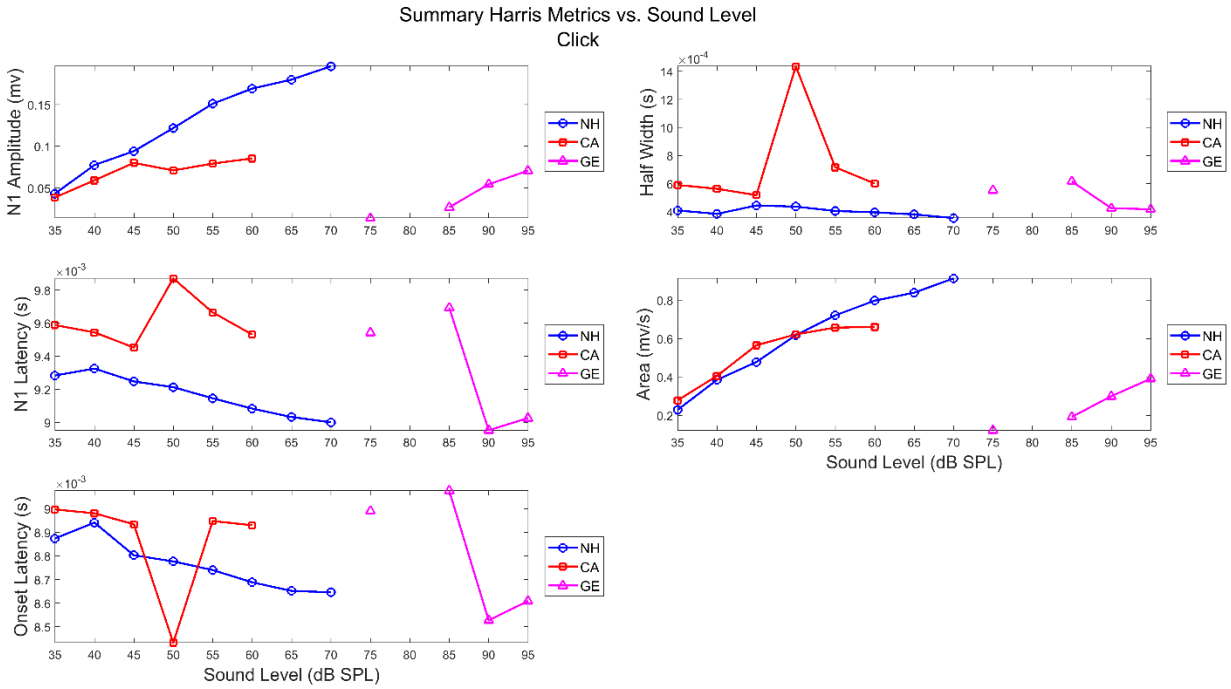

Figure 4.5 Example of the averaged Harris metrics for the three different hearing types plotted versus sound level. The different line colors correspond to the different hearing types.

4.2 Onset Response Metrics

As mentioned in section 2.3 above, clicks do not have a steady state response and thus the SP and CM were not calculated for any of the click responses. However, clicks do have an onset response, and an onset SP and onset CM can be calculated. Since these metrics were calculated at the same time as the Harris metrics, only the sound levels above threshold were measured. For the onset SP, it was identified as the positive peak before the onset of N1. If no actual peak was identified, the onset SP was set as the max in the onset window before the onset of N1. To calculate the onset CM, the positive and negative polarity averages from before were used. The onset CM was calculated within the first one ms after the start of the onset window, and was the average of the differences between the maximum and minimum points for the positive and negative polarities. These metrics were also plotted on the response waveforms with the Harris metrics, as shown in Fig. 4.1 above. Once the metrics were calculated for all of the relevant sound levels, they were plotted versus level as shown in Fig. 4.6 below. As with the initial and Harris metrics, after the individual metrics were calculated, they were averaged within the same chinchilla, and then the

metrics were averaged in chinchillas with the same hearing type, with a final plot showing the three different hearing types together as shown in Figs. 4.7-4.9 below.

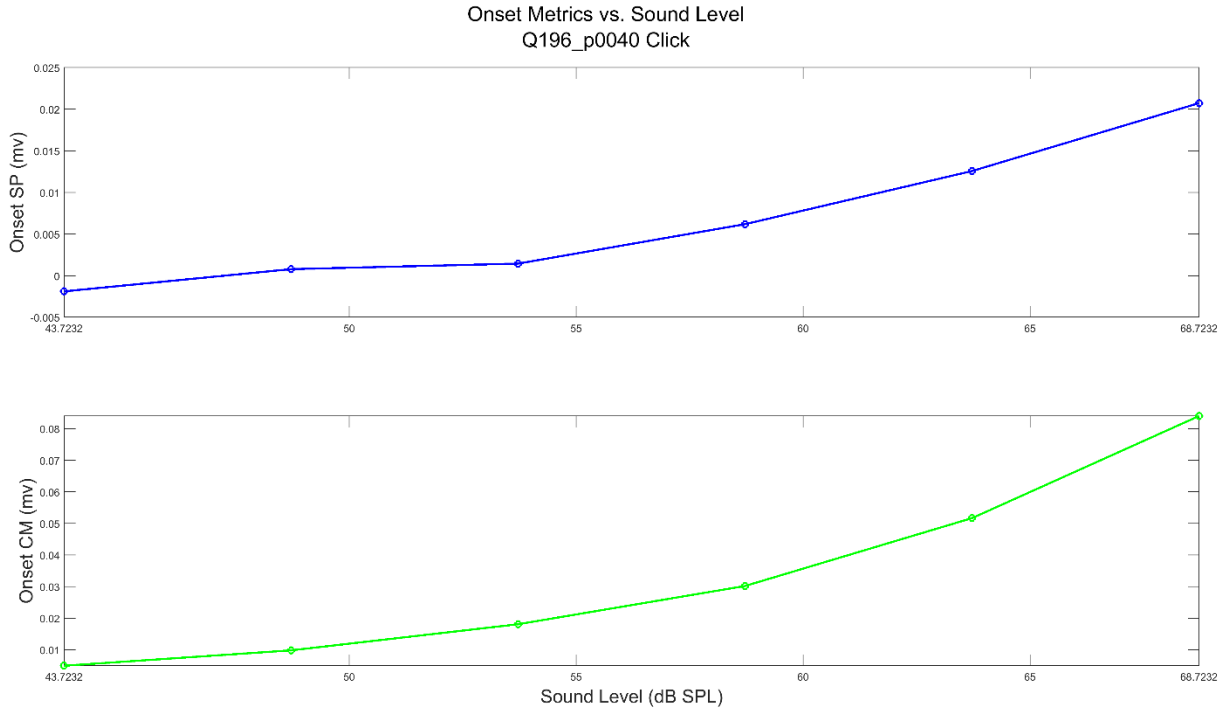

Figure 4.6 Example of the onset metrics plotted versus sound level for click responses from a single data file in a NH chinchilla (Q196). The line colors correspond to the line colors of the metrics on the waveform plot in figure 4.1 above. Note that the responses here, especially the SP, are more clean and consistent with the onset metrics compared to the standard metrics calculated in Fig. 2.4 above. Also note that the maximum amplitude of the SP is larger here than for the standard metric calculated in Fig. 2.4 (0.025 mv vs. 0.01 mv respectively).

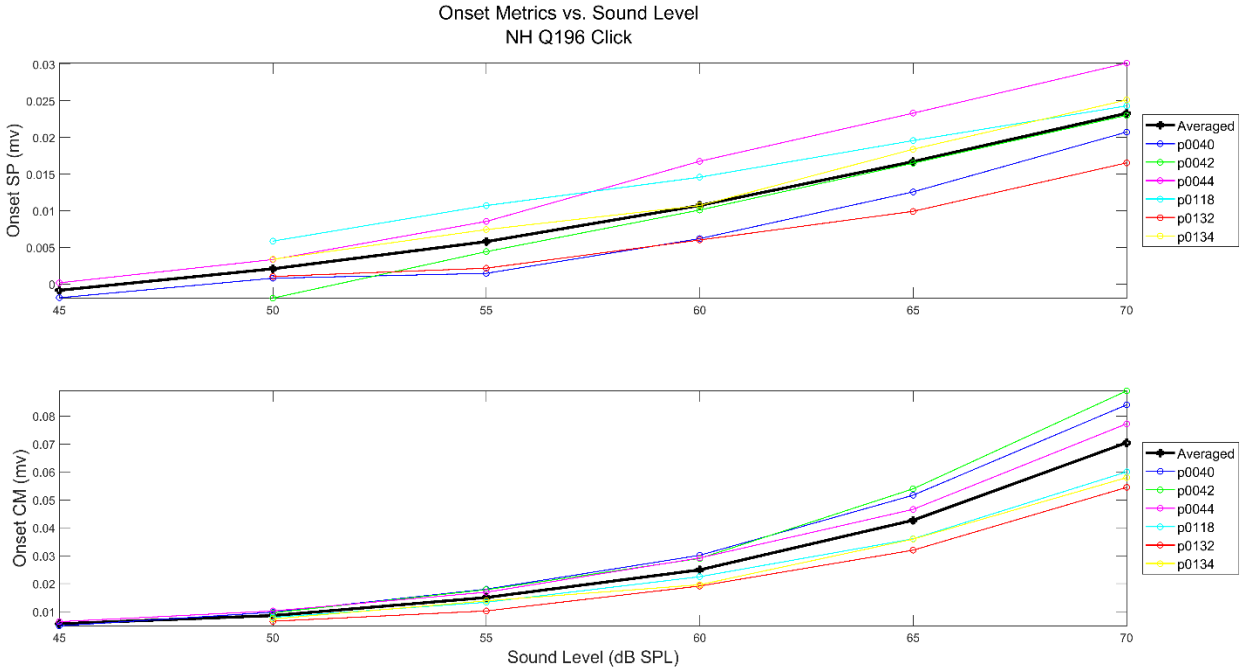

Figure 4.7 Example of the onset metrics averaged across data files for one chinchilla. The thicker black lines represent the averaged metrics and the colored lines correspond to the different data files. Note that the onset metrics calculated here are more clean and consistent compared to the standard metrics calculated in Fig. 2.9 above which had more variability between data files. Also note the maximum amplitude of the SP is larger here than for the standard metric.

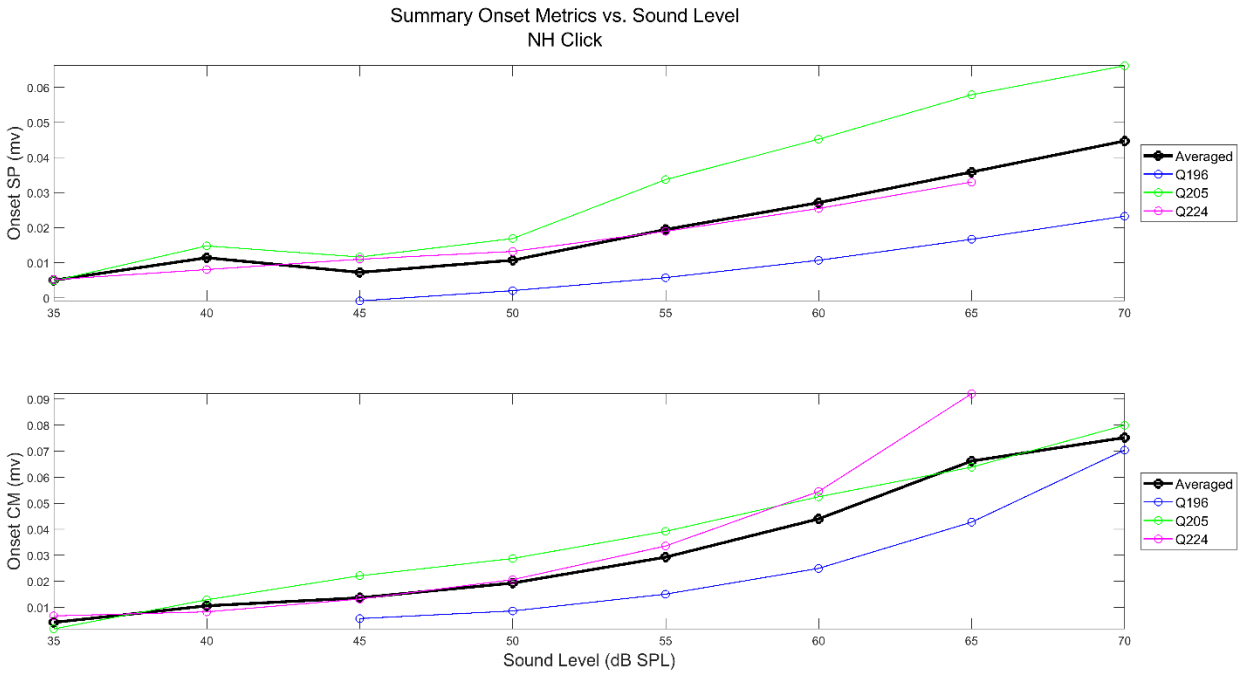

Figure 4.8 Example of the onset metrics averaged across chinchillas with the same hearing type (NH). The thicker black lines represent the averaged metrics and the colored lines correspond to the different chinchillas with the specified hearing type. Note that there is less variability across animals with the same hearing type for these onset metrics compared to the standard metrics calculated in Fig. 2.11 above.

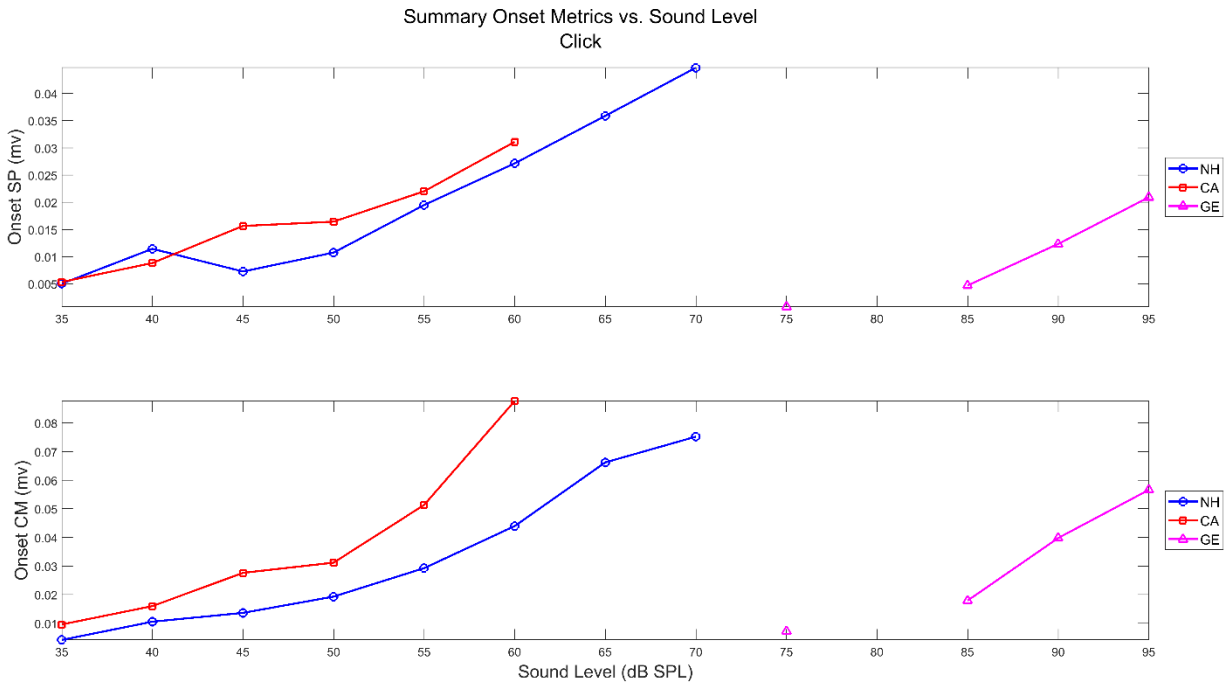

Figure 4.9 Example of the averaged onset metrics for the three different hearing types plotted versus sound level. The different line colors correspond to the different hearing types. Note that the trend of these metrics is more consistent across sound level than for the metrics calculated in Fig. 2.13 above, especially for the SP metrics.

4.3 Discussion

Most of the Harris metrics were consistent and changed as expected with changing sound level. Both the peak amplitude and the area of the response increased with increasing sound level. This is as expected because increasing sound level elicits a stronger response which would increase the amplitude and area of the response. The peak latency also consistently decreased with increasing sound level. Again, this is as expected because louder sound levels elicit a clear response sooner and thus the latency of the peak would decrease, or shorten. The onset latency was fairly consistent in decreasing with increasing sound level, but it was not as consistent as the peak latency. The half-width metric was the least consistent, in part because it relied on the onset and peak latencies and the onset latency was already not as consistent.

The onset SP and the onset CM were consistent across chinchillas and hearing types as well. Both metrics were well defined and relatively easy to identify and calculate. For all but a few minor exceptions, both metrics increased with increasing sound level. This is as expected since a louder sound level elicits an overall stronger response. It should also be noted that these onset

metrics were more consistent and less variable, especially the onset SP, compared to the standard metrics calculated in Chapter 2 above, both within the same animal across data files and across animals with the same hearing type. A comparison of the standard metrics and the onset metrics is shown in Fig. 4.10 below. As a reminder, the SP was filtered out in the initial data recording setup which accounts for the smaller standard SP response in the top panel of the figure below. The onset SP however has a consistent trend across sound level with a larger amplitude even though it's from the same filtered data, meaning the onset SP metric is a way to get decent SP data even with high cutoff frequencies which is not possible with the standard SP metric.

When comparing the averaged Harris metrics across hearing type, there were a few trends that were identified. The first was how the N1 amplitude changed with the different hearing types. As seen in Fig. 4.5 above, NH had the largest amplitude that steadily increased with sound level. The amplitude for CA chinchillas followed NH at levels close to threshold, but eventually plateaued at a lower amplitude. The amplitude for the GE chinchillas was much lower compared to NH. Similar to the N1 amplitude, the GE chinchillas had a much lower total area compared to NH and CA. Also similarly, the CA chinchillas generally followed the trend of the NH area at levels close to threshold, but plateaued at a lower total area than the NH chinchillas. Another trend was in the latencies across hearing type, specifically between CA and NH. For both the N1 and onset latencies (with the exception of one level), the CA chinchillas had slightly increased latencies compared to the NH chinchillas.

For the averaged onset metrics across hearing type, there were also a couple of trends that arose. The first was the onset SP where the GE chinchillas had a lower value for much higher sound levels as seen in Fig. 4.9 above. The CA chinchillas generally followed the NH onset SP trend, but with consistently slightly higher values. This trend in the CA chinchillas is not as expected since carboplatin damages the IHCs and the SP is thought to mainly be the response of the IHCs, but the reason for this trend here is unknown. Similarly, for the onset CM, the GE chinchillas had lower values at higher sound levels and the CA average followed the trend of the NH average, though with more pronounced consistently higher values over the NH compared to the onset SP. These trends for both the Harris and onset averaged metrics could offer more ways to identify hearing loss and potential sources of the loss in addition to the initial metrics described in Chapter 2.

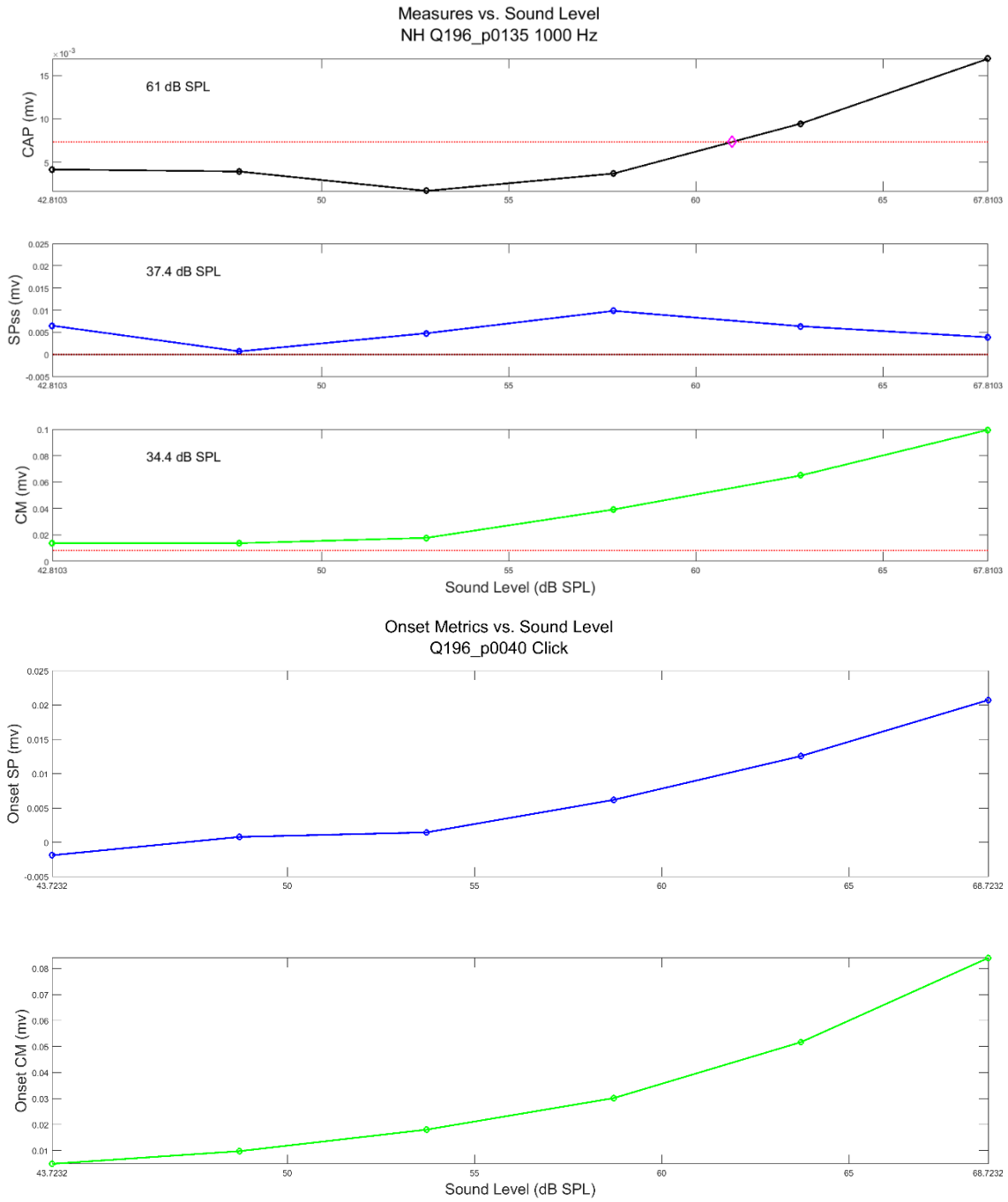

Figure 4.10 Comparison of trends and variability in metrics between the standard SP and CM from Chapter 2 and the onset SP and CM. The figure on the top shows the standard SP in blue and the standard CM in green. The figure on the bottom shows the onset SP in blue and the onset CM in green. The onset CM has a comparable, consistent trend like the standard CM metric. The onset SP however has a more consistent, less variable trend compared to the standard SP metric despite still being bandpass filtered.

5. GENERAL DISCUSSION AND FUTURE DIRECTIONS

5.1 Robustness of Individual Metrics

As mentioned in the Chapters 2 and 4 results above, the individual metrics were sensitive and accurate enough to identify trends between different hearing types. The original three metrics of CAP, SP, and CM were able to identify a clear hearing loss in the GE chinchillas which was consistent with the damage to the OHCs identified in the initial data collection. The additional Harris and onset metrics were also consistent and able to identify hearing loss in the GE chinchillas with decreased amplitudes and areas as well as decreased onset SP and CM. There were also consistent trends between the CA and NH responses for the Harris and onset metrics. For the Harris metrics, the CA chinchillas generally followed the trend of the NH average, especially at sound levels close to threshold, but plateaued at values below the peak NH values for the same sound levels. As for the onset metrics, the CA chinchillas again generally followed the trend of the NH averages, though with slightly higher values compared to the NH responses.

5.2 Evidence That Awake Responses Are Physiological in Nature

There were a few criteria that were used to support that the awake responses are physiological in nature, meaning that the responses are not an electrical artifact. One of the criteria to support that the awake responses are physiological is that the latency of the response decreases as the sound level increases, and this is based on cochlear response properties. As shown in figures 3.28-3.32 and 3.35 above, as the attenuation level decreased, or as the sound level increased, the latency of the CAP response decreased. This was true for click and tone burst responses, suggesting that the recorded responses are physiological in nature.

Another criteria to support that the awake responses are physiological is that higher frequencies have shorter latencies at the same sound level, especially at sound levels closer to threshold. Because the cochlea is tonotopic and higher frequencies are near the base, sound reaches higher frequencies first and thus the latency of the response is shorter. Also, at suprathreshold sound levels, there is a tendency for the sound to activate higher frequencies along with the frequency specific to the stimulus, meaning that at higher sound levels the lower frequencies may have the same latency as higher frequencies due to cochlear dispersion. Both of these cases are

seen in the awake responses. For both the response at a sound level well above threshold and the response at a sound level closer to threshold, shown in figures 3.36 and 3.39 above respectively, it is clear that the higher the frequency is, the shorter the latency. This further suggests that the awake responses are physiological in nature.

One final criteria to support that the awake responses are physiological is that the threshold for a clear response is within an expected range of sound levels for thresholds in normal hearing chinchillas. Thresholds for a clear ECochG response in a normal hearing chinchilla are usually around 25 – 35 dB SPL, an example of which is shown in Fig. 2.3 above where the threshold determined based on round window recordings for a normal hearing click response was 37.6 dB SPL. In the awake procedures, the threshold was generally around 70 dB attenuation for click responses, as shown in figures 3.24 and 3.29 above. Based on the inverse calibration in Fig. 3.16, the maximum sound level for clicks is about 105 dB SPL, meaning that 70 dB attenuation is around 35 dB SPL, which is just within the expected range for normal hearing chinchillas, suggesting that the awake responses are physiological in nature.

5.3 Reliability of Electrocochleography Measures from the Ear Canal

In order for it to be feasible to record non-invasive ECochG responses from the ear canals of awake chinchillas, the responses recorded must be reliable. As mentioned in the previous section, one check that the responses are reliable is that they are physiological in nature which was confirmed above. Another check that the responses are reliable is that they are repeatable across time. In order to check this, the same responses were collected from a couple of chinchillas in different weeks.

5.3.1 Repeatability

There were two chinchillas whose same responses were collected in different weeks. The response comparisons are shown in Figs. 3.39-3.42 in the test/retest section of Chapter 3 above. From the figures it is clear to see that the actual ECochG response (e.g., the CAP shape) was repeatable across time. Though the baseline noise levels differed due to the very wide filter (0.3 – 3000 Hz), the response waveform stayed the same. This repeatability of response indicates that the ECochG responses from the ear canal of awake chinchillas are reliable.

5.3.2 Number of Repetitions Required

Because there is inherently more noise in awake, non-invasive ECochG recordings, the number of repetitions, or sets of each polarity of a stimulus presented, needs to be increased. However, increasing the number of repetitions collected for a given stimulus condition increases the amount of time it takes to collect one condition. This then inherently limits how much data can be collected in one awake procedure (~45 minutes of data collection). To help determine the minimum recommended repetitions needed to produce a clear response, we collected a large number of repetitions (3,000 reps) and performed bootstrapping. As shown in the figures below, a minimum of at least 1,000 reps is required to collect clear, consistent responses, though increasing to at least 2,000 reps is better. Additional signal processing or artifact rejection techniques may be able to be developed in future work to reduce the number of repetitions and optimize data collection.

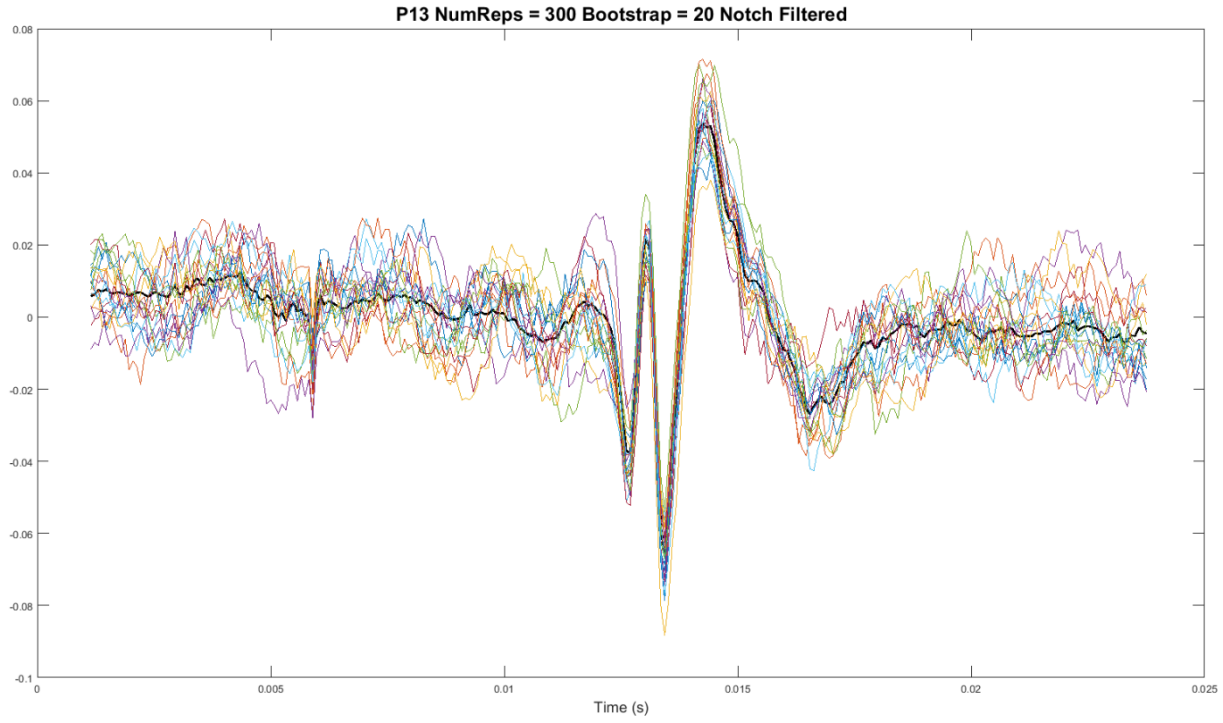

Figure 5.1 Example of bootstrapping for a click response. 300 sets of positive and negative polarities were randomly chosen from the total 3,000 sets 20 different times, and each of these 20 averages were notch filtered at 60 Hz. The different line colors represent the filtered average of the polarities for each of the 20 bootstrap repetitions. The thick black line is the actual 3,000 set filtered averaged response.

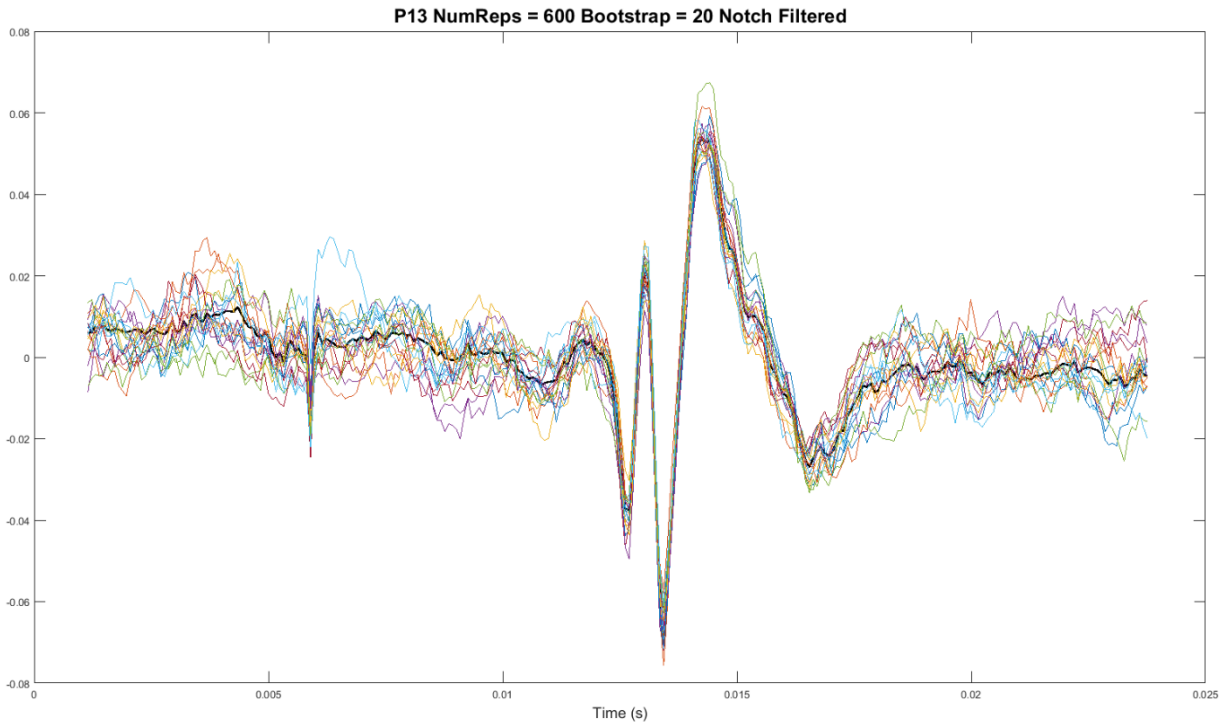

Figure 5.2 Example of bootstrapping for a click response with 600 sets of each polarity randomly chosen from the total 3,000 sets. The 600 sets were chosen 20 different times and then notch filtered, and the different line colors represent the filtered average of the polarities for each bootstrap repetition. The thick black line is the actual 3,000 set filtered averaged response.

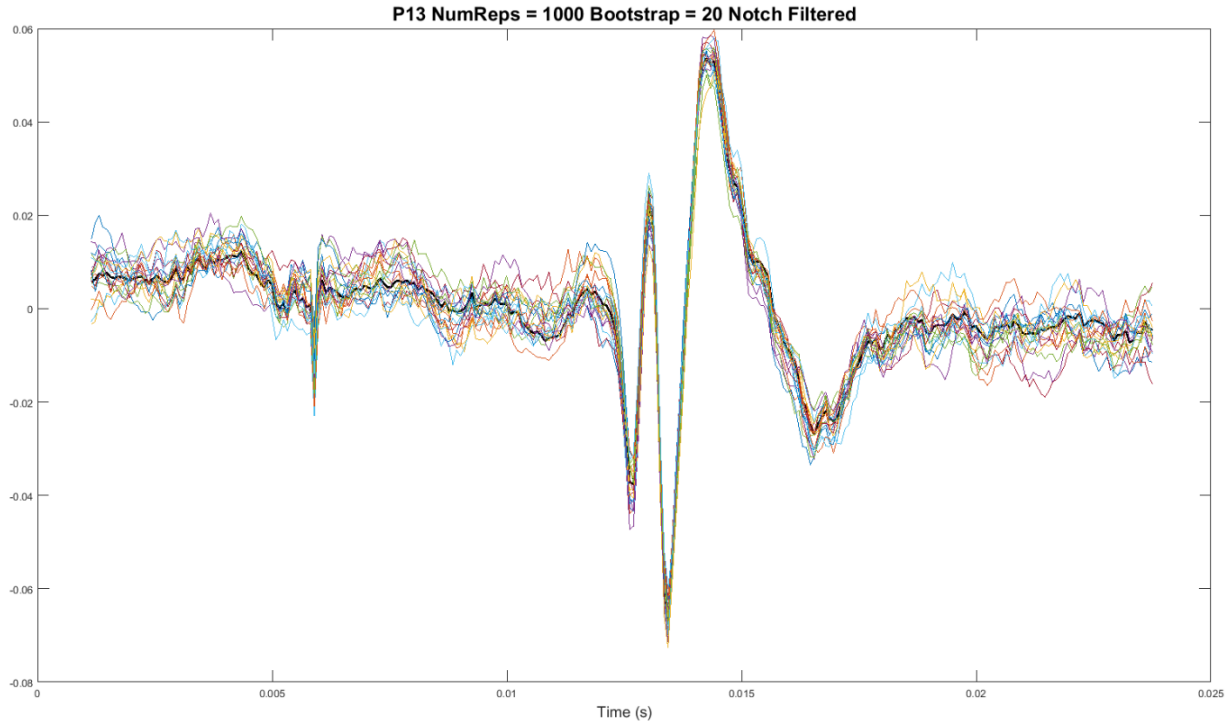

Figure 5.3 Example of bootstrapping for a click response with 1,000 sets of each polarity randomly chosen from the total 3,000 sets. The sets were chosen 20 different times and then notch filtered, and the colored lines represent the filtered average of the polarities for each of these bootstrap repetitions. The thick black line is the actual 3,000 set filtered averaged response.

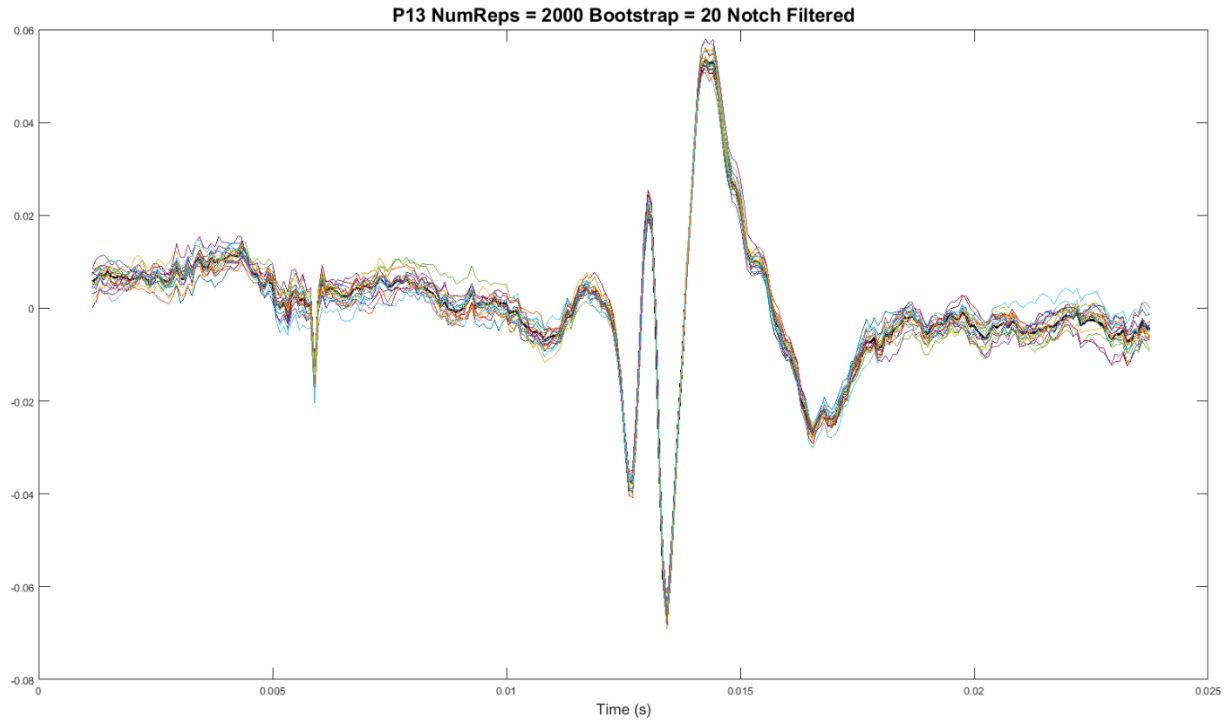

Figure 5.4 Example of bootstrapping for a click response with 2,000 sets of each polarity randomly chosen from the total 3,000 sets. The sets were chosen 20 different times and then notch filtered, and the colored lines represent the filtered average of the polarities for each of these bootstrap repetitions. The thick black line is the actual 3,000 set filtered averaged response.

5.3.3 Potential Artifacts

One of the potential artifacts from awake recordings is noise from cable and wire interference. We found that it was especially helpful and important to twist or braid the three wires from the three different electrodes. We also found that separating the cables for the two different outputs of the voltage follower helped reduce noise. Another potential artifact comes from the tiptrodes themselves. Because they are wrapped in gold-foil, any time they are handled to be placed in the ear canal, the foil starts to wear off. If enough foil wears off, a clean signal is not able to be picked up. Furthermore, if the tiptrode is not placed in the ear canal well and there is not a good connection between the tiptrode and the ear canal, the signal collected may not be strong enough and could cause the pre-amplifier to go into an overloaded state, which may add artifact. On a side note, it does appear sometimes that it can take ~5 minutes or so for the noise levels to reach a minimum (presumably as the gold-foil/ear canal interface stabilizes).

5.4 Future Work

The first next step is to perform more awake ECochG recordings from more normal hearing chinchillas. Due to the COVID-19 pandemic, the amount of awake data collected was limited not only in the number of normal hearing animals tested, but in the type of responses collected as well. Ideally, a range of attenuation levels for clicks and different tone frequencies would be collected. In order to obtain relatively clean data in the time available, the number of sets of polarities presented during most awake procedures erred on the higher side (i.e., 3,000 sets) which takes longer to collect and thus limits the amount of different responses able to be collected in one session. In the future, data collection would be optimized to collect the minimal sets of polarities necessary to obtain clean data – possibly with an adaptive noise criterion that stops collecting when the averaged response is below a certain threshold – while also interleaving attenuation levels and frequencies to make collection more efficient.

Another next step would be refining data collection in terms of equipment used to collect data. Even with the steps taken to reduce noise, there was still a decent amount of lingering noise in the awake ECochG responses. One potential step would be to optimize the circuitry between the output of the tiptrodes and the input to the computer to further reduce noise. This could potentially be accomplished by using better, more advanced equipment like the Biosemi Active Two which has internal filters and wiring that greatly reduces noise (e.g., DRL and CMS circuitry). Another potential step would be to fine tune the filtering on the SR 560 pre-amplifier to allow for the passing of a SP response while still limiting the amount of excess noise.

One final next step would be to refine the post-processing of the data. A basic notch filter at 60 Hz was used here for most of the ear canal ECochG responses, but as mentioned in Chapter 3, though this helped eliminate some noise, it is by no means perfect or ideal for all responses. Ideally, the frequency spectrum of the responses would be calculated and filtering would be adjusted based on this. This would mean for example that if there was more noise at 180 Hz and 360 Hz as well as at 60 Hz, then a comb filter could be used to remove all of these noise sources rather than just addressing the 60 Hz noise.

APPENDIX A. ADDITIONAL FIGURES

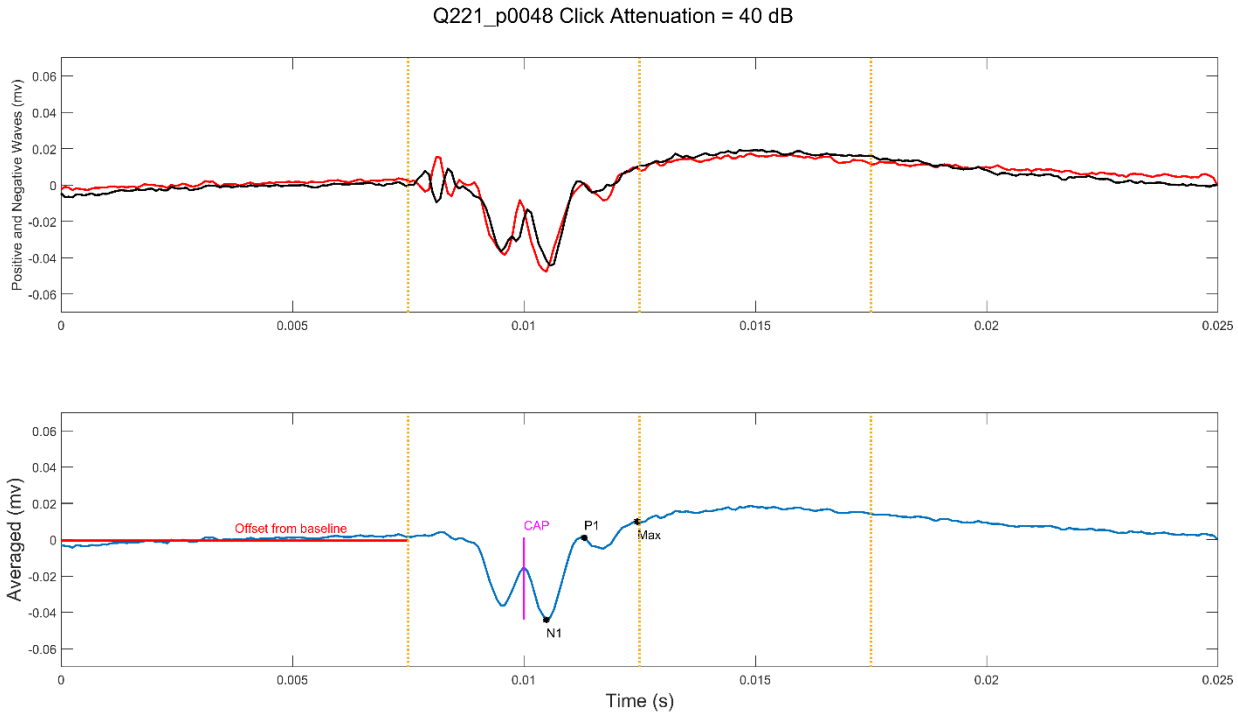

Figure A.1 Example of a click response from a CA chinchilla. Note that there are two negative peaks and N1 was chosen as the largest negative peak in all cases.

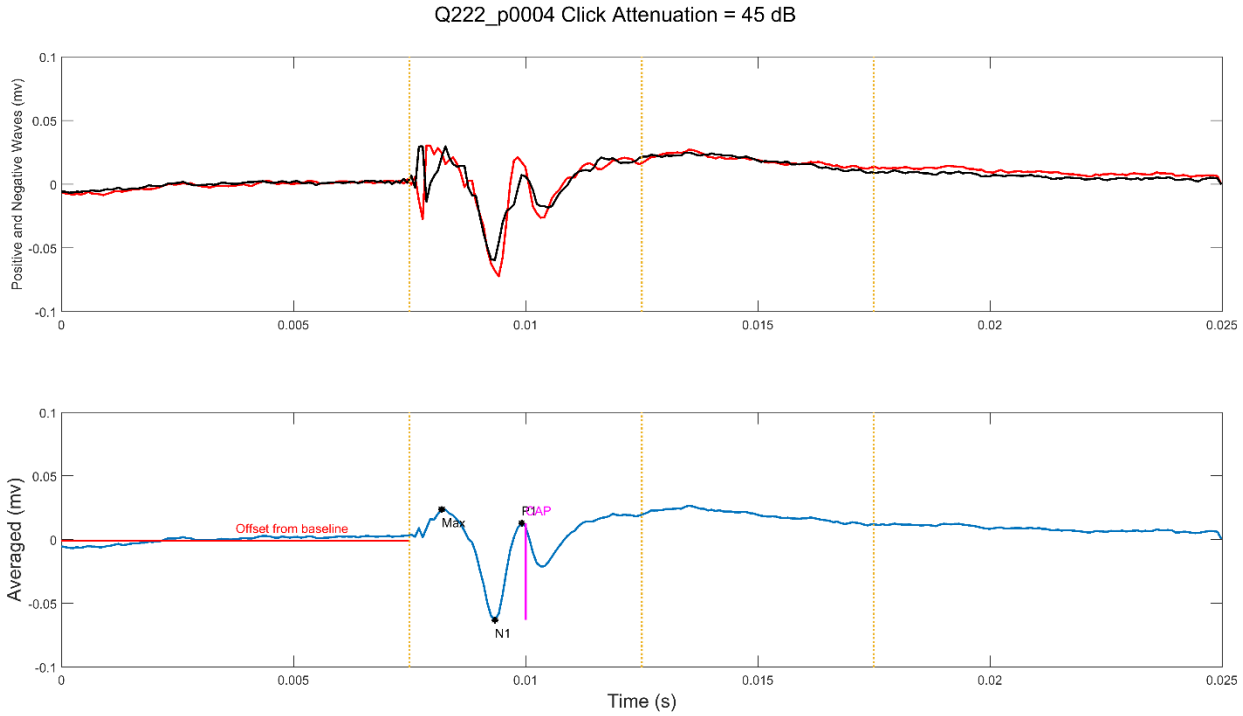

Figure A.2 Example of a click response from a different CA chinchilla. Note that there are two negative peaks again. Also note the large onset SP and CM.

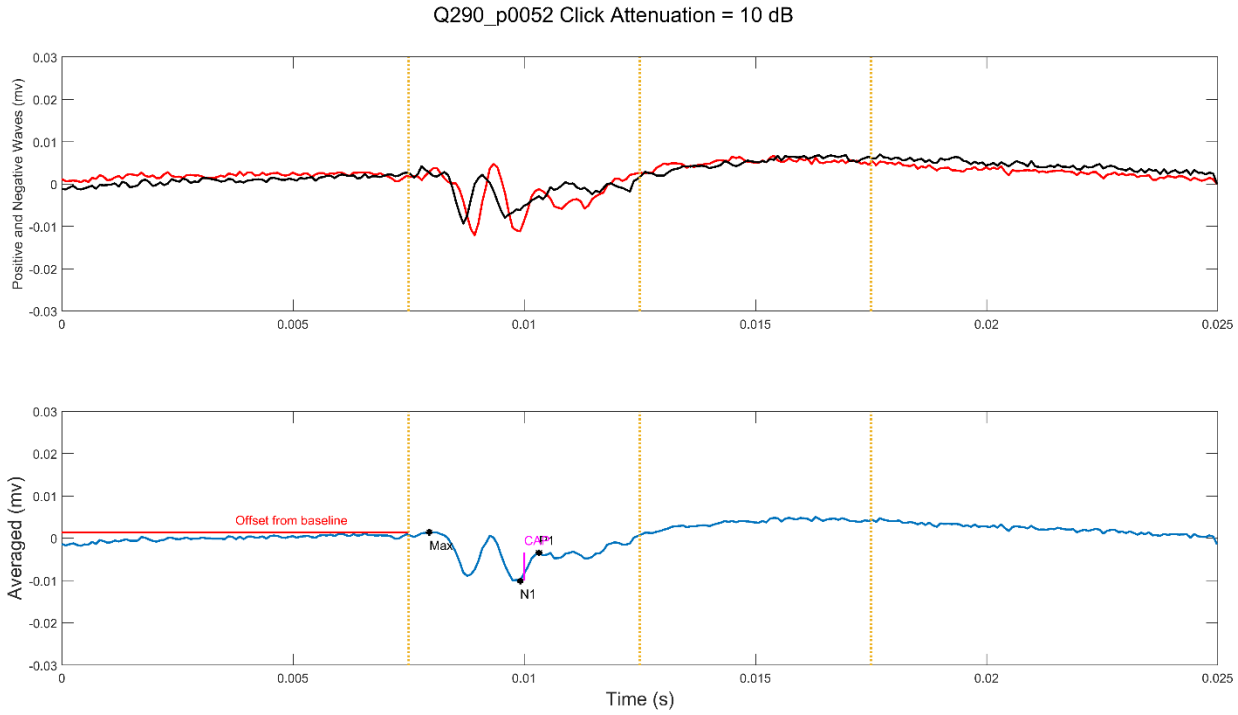

Figure A.3 Example of a click response from a GE chinchilla. The attenuation level is much louder compared to the NH and CA chinchillas due to overall hearing loss from the gentamicin. Note there are two negative peaks and N1 was chosen as the largest negative peak. Also note the different response shape after the CAP.

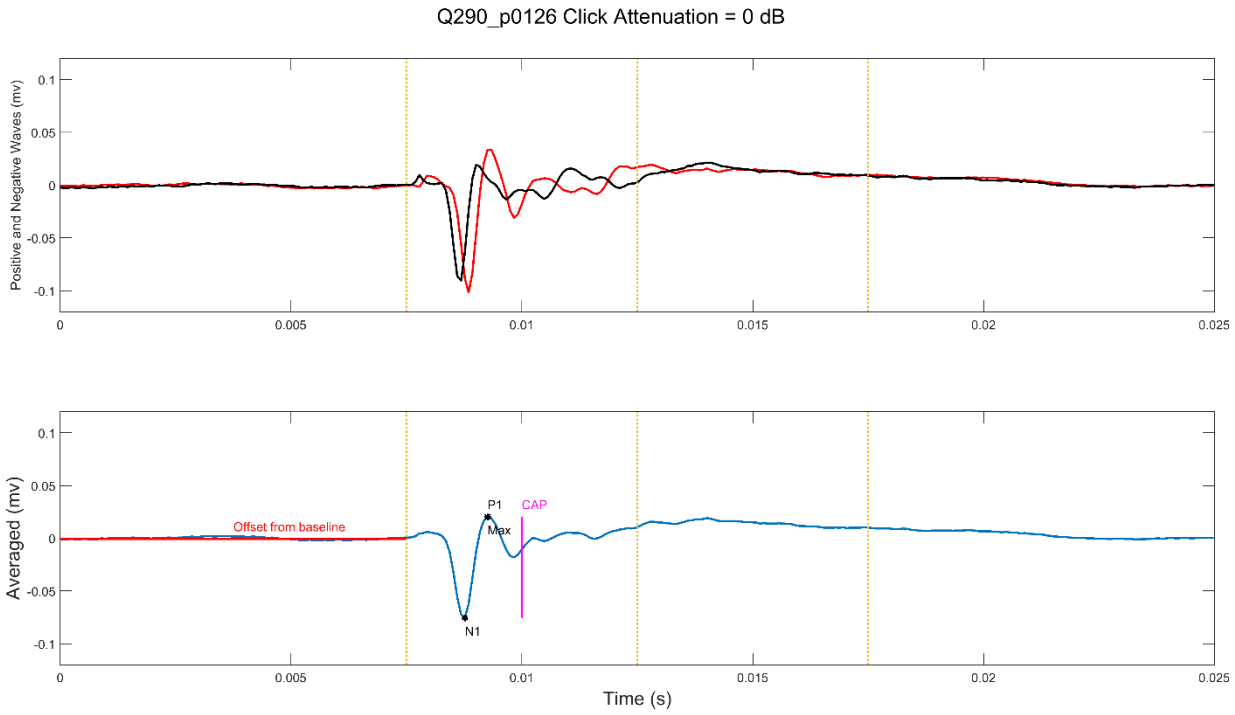

Figure A.4 Example of a different click response from the same GE chinchilla. The attenuation level is much louder compared to the NH and CA chinchillas due to overall hearing loss from the gentamicin. Note the different response shape after the CAP.

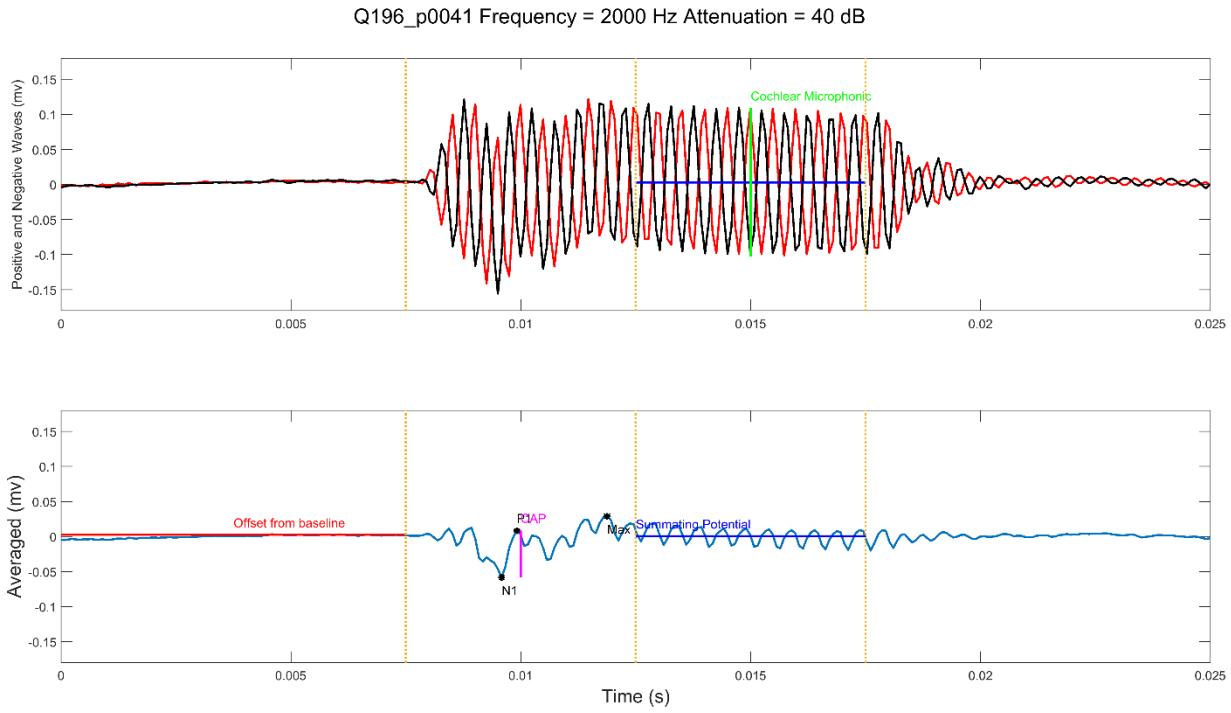

Figure A.5 Example of a 2 kHz tone response from the same NH chinchilla as the click examples shown in Chapter 2. Note the lingering microphonic in the averaged response on the bottom subplot.

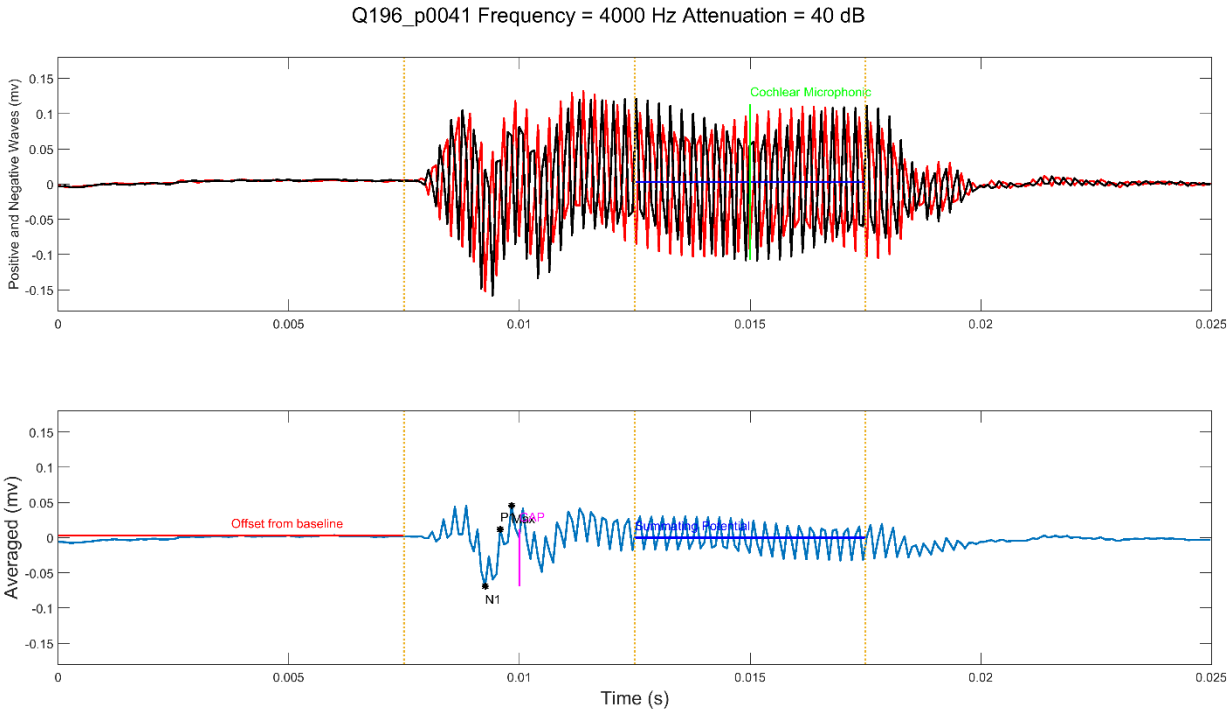

Figure A.6 Example of a 4 kHz tone response from the same NH chinchilla as the click examples shown in Chapter 2. Note the lingering microphonic in the averaged response on the bottom subplot which has affected where P1 is determined and thus the amplitude of the CAP.

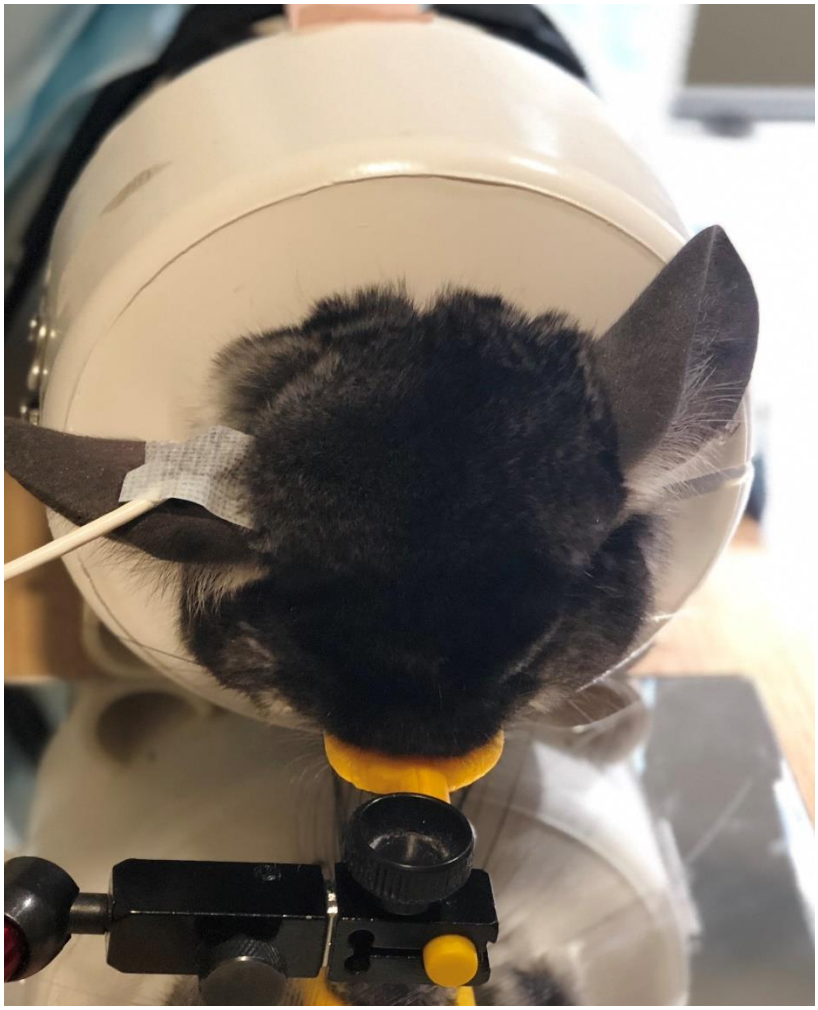

Figure A.7 Example of a chinchilla in the awake restraint tube and nose holder.

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