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Kaitlyn M. Kennedy

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**SPATIAL IRREGULARITIES OF COMPOUND ACTION POTENTIAL
ORIGINATION IN INDIVIDUAL COCHLEAE**

A Doctoral Thesis

Presented to

The Graduate College of
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Doctor of Audiology

By

Kaitlyn M. Kennedy

May 2016

SPATIAL IRREGULARITIES OF COMPOUND ACTION POTENTIAL ORIGINATION IN INDIVIDUAL COCHLEAE

Communication Sciences and Disorders

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Doctor of Audiology

Kaitlyn M. Kennedy

ABSTRACT

The present study aims to evaluate the origin of compound action potential (CAP) responses in the guinea pig ear using the following method. Pipettes were sealed into the cochlear apex of 26 guinea pigs to inject ototoxic pharmaceuticals that inhibit nerve responses without affecting cochlear function. The rate of the injection was adjusted once each minute to account for variations in the diameter of the cochlea and to maintain a steady flow of pharmaceuticals. CAP measurements collected at around 90-second intervals using tone burst stimuli during the injections allowed evaluation of the neural responses over time. Responses to higher stimulation levels revealed a later ablation time, indicating they originate from a more basal, higher frequency region than 2 kHz. CAP abolition rates revealed variations between individual ears. Additionally, distortion-product otoacoustic emission (DPOAEs) measurements were collected to evaluate cochlear response. Findings showed that the injection did not alter DPOAE response amplitudes, indicating it had no effect on cochlear mechanics. These findings provide insight into irregular spatial origins of cochlear responses, and therefore an irregular frequency-place map, in individual ears.

KEYWORDS: compound action potential, frequency-place map, kainic acid, apical injection, otoacoustic emissions

This abstract is approved as to form and content

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INTRODUCTION

The human auditory system has received a great deal of attention in recent history; however, cochlear and neural origins of various electrophysiologic responses are still being reviewed. The compound action potential (CAP) persists as one of the most common electrophysiologic responses used to extrapolate information about the cochlea, and can assist in establishing hearing thresholds, examining VIIIth nerve responses to sound, evaluating cochlear tuning, or determining generation sites of other measures (Lichtenhan, 2012). CAPs provide invaluable information for both basic and clinical science due to their frequency specificity at varying intensity levels and rates.

One of the early studies evaluating CAP origins came from Kiang (1965). His lab compiled six years of experiments in order to provide information on the neurophysiology of hearing. Specifically, they wanted to examine single auditory nerve fiber (ANF) responses to clicks, tone bursts, and complex sounds. In order to study ANF responses, Kiang (1965) prepared 50 cats in good health, placing a “gross electrode” near the round window and a micropipette electrode in an ANF underneath the cerebellum where the VIIIth nerve exits the internal auditory meatus. Post-stimulus time histograms (PST) or interspike interval histograms obtained from each nerve fiber facilitated display and analysis of the collected data. When Kiang compared PSTs to the averaged round window gross electrode recordings, which relate to wave I of an auditory brainstem response (ABR; Berlin & Hood, 2009), the latencies of the single fiber and gross recordings correlated, indicating the gross waveform is the sum of ANF responses to a stimulus (Kiang, 1965). This finding indicates CAP latency responses recorded from the

round window correlate to ANF responses collected from single units. Therefore, a change in the latency of a CAP response represents a change in the synchronous discharge of single unit ANF. This conclusion correlates with the findings of Taberner and Liberman (2005), who reported that CAP tuning curves elicited by tone-burst stimuli correspond well to individual ANF responses. Miller, Abbas, and Rubinstein (1999), who modeled CAP tuning curves in cats based on ANF responses, also verified this finding. In a similar study, Dolan, Mills, and Schmiedt (1985) evaluated the tuning of both brainstem responses and CAPs using forward masking techniques in gerbils and compared them to ANF tuning curves. When compared, all the tuning curves had a similar shape, frequency response, and varied similarly as a function of frequency. Ultimately, this indicates that peripherally, CAP tuning curves have the ability to show frequency specificity. For the present study, CAP responses must show frequency specificity so the spatial origins of frequency specific responses can be accurately evaluated.

Multiple studies have evaluated CAP responses at low levels. They found CAPs have similar responses to single unit measures at low intensities (Özdamar & Dallos, 1976; Teas, Eldredge, & Davis, 1962) and activate a frequency specific group of ANFs along a small, frequency specific portion of the cochlea (Cheatham, Naik, & Dallos, 2010). Therefore, as the intensity of a stimulus decreases, the frequency specificity of the response increases.

Derived band masking responses allow the evaluation of CAP waveforms from a specific region of the cochlea, leading to frequency specific measures (Earl & Chertoff, 2012; Eggermont, 1976; Elberling, 1974; Evans & Elberling, 1982; Shore & Nuttall,

1985; Teas et al., 1962). In 1976, Eggermont evaluated the effects of intensity on the auditory brainstem response using derived bands. He utilized high pass masking to evaluate the size of the area activated by a 2 kHz tone burst. Eggermont (1976) found the activation area grew as the intensity increased (Figure 1). For example, at 25 dB SL, one octave of the cochlea from 1-2 kHz was active, while at 70 dB SL, areas on the basilar membrane from .7 kHz to the high frequency limits of the cochlea contributed to the response. This means that at a higher intensity, more of the basilar membrane (i.e. broader activation) contributes to a CAP response than at a lower intensity. Specifically, Eggermont found intensities greater than 50 dB SL receive more contribution from higher frequency, basal places on the basilar membrane than at lower intensities. Additionally, when response areas are compared to ANF tuning curves (Eggermont, 1976; Liberman & Dodds, 1984), the same shape is visible, with a peak at the central frequency and a high-frequency tail.

Apical injections remain useful when using pharmaceuticals to evaluate cochlear origin since there is reduced loss of drug to other sources and more uniform treatment of the pharmaceutical throughout the cochlear partition (Lichtenhan et al., 2014). With drug delivery from any location, the drug experiences liberation from dosage form, absorption into the cochlea, distribution throughout the cochlea, metabolism, and elimination from the ear through various modalities like blood (Salt & Plontke, 2009). With round window delivery, variability exists in the amount of drug reaching multiple parts of the cochlea due to variations in round window permeability from experimental manipulations (Hahn, Kammerer, DiMauro, Salt, & Plontke, 2006; Höft, 1969; Mikulec, Hartsock, & Salt, 2008), dispersal to other compartments (ex:

Ilberg & Vosteen, 1969), abolition from blood (Shepherd & Colreavy, 2004; Rask-Andersen, Schrott-Fischer, Pfaller, & Glueckert, 2006), and loss to the cochlear aqueduct (Salt, Hale, & Plonkte, 2006). Apical injections allow for more consistent, controlled drug concentrations throughout the cochlea (Salt et al., 2006) and determination of specific response regions to various stimuli and electroacoustic measures (Lichtenhan et al., 2014; Salt et al., 2013).

Ultimately, apical injections will assist in our comprehension of otoacoustic emission (OAE) origins since they are not understood on the same level as neural measures at this time. To date, OAEs have allowed non-invasive measures cochlear tuning (Shera, Guinan Jr., & Oxenham, 2010; Shera & Guinan Jr., 2003; Charaziak, Souza, & Siegel, 2013), studies of forward and reverse propagation in the cochlea (Shera & Guinan Jr., 2003; Rasetshawane, Argenyi, Neely, Kopun, & Gorga, 2013; Neely, Norton, Gorga, & Jesteadt, 1988), test models of emission generation (Lichtenhan, 2012; Charaziak & Siegel, 2014), and hypothesis on spatial origins within the cochlea (Shera, 2015). It is thought that by collecting CAP and OAE measures in the same way, a comparison between responses will help further our understanding of the origins of OAE responses. In the present study, we use serial CAP measurements taken during an apical injection to show that classically understood origination regions are correct, but vary among ears.

METHODS

Animal Preparation

The Animal Studies Committee at Washington University approved this study under protocols 20120113 and 20100135. Experiments used National Institutes of Health strain-pigmented guinea pigs of either sex who weighed 400-600 g. Guinea pigs ($n = 26$), around the same age, were initially anesthetized with 100 mg/kg of sodium thiobutabarbital, followed by ~ 1% isoflurane in oxygen delivered through artificial ventilation by way of a tracheostomy. The respirator was set to maintain end tidal CO₂ levels at 5%. Heart rate, O₂ saturation, and expired CO₂ levels were monitored with a pulse oximeter (CapnoTrue AMP, Bluepoint Medical, The Netherlands). A rectal thermometer and DC-powered heating blanket assisted in maintaining body temperatures at 38°C. The right cochlea was accessed using a ventral approach through the auditory bulla. A hollow ear bar with a place for both CAP stimuli and a distortion product OAE (DPOAE) probe was placed in the ear canal. Before electrical recordings began, Pavilon was injected intravenously to suppress middle ear muscle contractions.

Controlled Injections

In order to inhibit neural responses, a pipette sealed in the cochlear apex of one of the guinea pigs injected kainic acid (KA, 2.16 mM) in artificial perilymph (Figure 2). Initial experiments used injections of tetrodotoxin (TTX, 250 ng/ml), but excitatory post-synaptic potentials (EPSP) still existed after ablation. In contrast, KA inhibited the EPSPs and was utilized for the remainder of the study. In order to seal the pipette in the

cochlear apex and create a hydrophobic surface, the mucosal covering was removed from the apex, the bone dried, a thin layer of cyanoacrylate glue put on the apex, and a two-part silicone (Kwick-Cast, WPI, Sarasota, FL) applied to create a bowl to retain any fluids. A $\sim 50\ \mu\text{m}$ fenestra made through the layers of glue and bone allowed a pipette tip ($25\text{-}40\ \mu\text{m}$) pulled from 1-mm glass tubing to be sealed in the cochlea. After inserting the pipette in the scala tympani, a wick removed the fluid droplet that formed at the site of the fenestra, and cyanoacrylate sealed the pipette in place. The pipette was attached to a 100- μL gas-tight syringe (1710TLL Hamilton) with a Plexiglas coupler (MPH6S10 World Precision Instruments, Sarasota, FL). A digitally controlled WPI Ultrapump (World Precision Instruments, Sarasota, FL) attached to the syringe regulated the pump speed, which changed once each minute ($25\text{-}626\ \text{nl/min.}$) to maintain a flow rate of $0.5\ \text{mm/min}$ throughout the widening area of the scala tympani. At the end of the injections, $10\ \mu\text{L}$ of KA solution was introduced to the cochlea.

The changes in intracochlear pressure created by injecting KA at varying rates are below those caused by respiration and the entry rate of cerebrospinal fluid at the cochlear aqueduct (Böhmer, 1993; Salt & DeMott, 1998; Salt, Gill, & Hartsock, 2015). Therefore, any changes noted in electrophysiologic recordings reflect the ototoxicity of the pharmaceuticals rather than a mechanical change in the cochlea. When using apical injections, CAP responses to low frequencies will ablate first. CAP responses at $40\ \mu\text{V}$ before the injection were used as a baseline. The intensity remained at the same level throughout the injection so a decrease in amplitude could be observed when the KA reached the cochlear origin of the response.

Electrophysiologic Measurements

An Etymotic Research ER-10C probe was connected to the end of the hallow ear bar. A custom written program (Microsoft Visual Basic) controlled stimuli generation and data collection through Tucker-Davis System 3 hardware. Stimuli were sent through TD-PA5 attenuators and TD-HB7 headphone amplifiers. Sounds were calibrated in one-quarter octave steps from .125 kHz to 22 kHz through the hallow ear bar. CAP differential recordings were collected between a platinum needle vertex electrode and Ag/AgCl ball electrode near the round window. An Ag/AgCl pellet electrode on the left side of the neck grounded the animal. An optically coupled amplifier (TD HB7, 1000x gain) with high and low pass filters, set at 5 and 15 Hz respectively, recorded signals. Digitization (48.8 kHz) and averaging were completed with TD-RP2 modules. In order to ensure the guinea pigs had normal hearing, CAP threshold curves with a 10 μ V criterion were completed from 1-22 kHz using an automated routine.

Responses were collected continuously, with a cycle of collections completed every 1 to 1.3 minutes. Throughout the course of injection, level series measured CAP amplitudes from 50-74 dB SPL or 43-73 dB SPL in 6 dB steps, at either 2 or 8 kHz respectively. Tone bursts had a two cycle rise/fall time using alternating polarity. Thresholds for both 10 μ V and 40 μ V criterion helped determine when responses ablated and create a baseline. Stimulus conditions were chosen so responses could fit in this time frame.

DPOAE Stimulus and Response Recordings

Acoustic stimuli were created by a Tucker-Davis (TD) system-3 hardware and controlled with a custom-written Visual Basic (Microsoft) program. The hollow earbar delivered two channels of stimuli (f_1 , f_2) to the ear canal through a modified Etymotic ER10C probe. Stimuli generated by two TD-RP2 modules passed through a TD-PA5 attenuator to control for level and were amplified by a headphone amplifier (TD-HB7). All stimuli and data came from a closed system. An ER10C microphone recorded emissions and calibrated the stimuli. Using a 70 dB SPL stimulus level in $\frac{1}{4}$ octave steps, calibration curves were created for each animal. Calibration data determined the intensities of all acoustic stimuli, measured in dB SPL. DPOAEs were obtained from .5-10 kHz using two tones at 65 dB SPL (L_1) and 55 dB SPL (L_2). Measurements were completed before, during, and after KA injections.

RESULTS

Origin of CAP is Variable Within and Between Animals

CAP amplitudes for a 2 kHz tone burst were collected at different intensities throughout the course of the injection. As the injection of KA progressed, amplitudes were slightly enhanced around 3 minutes and ablated after the enhancement, with the higher level CAPs ablating later than lower level responses. Figure 3 provides an example of CAP responses in relation to intensity and time after the injection. Since lower intensities were eliminated first with the apical injection, their origins are likely more apical and specific on the basilar membrane. High intensities were eliminated later and therefore likely have origins, which are more basal and widespread in nature. In Figure 3, the difference in time of ablation between the 50 dB SPL and 74 dB SPL responses is nearly seven minutes, although this difference varied across animals.

Although each animal's CAP responses ablated with KA, irregularities were noted between cochleae. Figure 4 demonstrates these variations with both KA (panels A-C) and TTX (panel D) for an 8 kHz level series. The spread of the response origin varied in each animal shown. In panel A, responses to higher intensities are ablated earlier than lower intensities, indicating a more apical origin and spread to lower frequency regions. Panels B and D display responses that have a more basal spread at higher intensity levels. Finally, Panel C represents responses that are ablated both early and late, indicating spread both basally and apically. Some of the variation of Panel C may be the result of the 80 and 90 dB SPL stimuli since the other panels have a high intensity of 73 dB SPL.

If the 80 and 90 dB SPL response are removed, the response spreads to higher frequencies similar to panel A.

Outer Hair Cell Function is Not Disrupted by Controlled Apical Injections

Since this study examined neural response ablation, the pharmaceuticals used in the injection needed to eliminate CAPs while leaving cochlear mechanics intact. TTX is known to block neural action potentials by binding to sodium channels (Lichtenhan et al., 2014) while kainate receptors are present both pre- and postsynaptically in inner hair cells (IHCs), possibly to respond to transmitters released by the IHCs during acoustic stimulation (Peppi, Landa, & Sewell, 2012; Fujikawa et al., 2014). The bonding of KA to these receptors inhibits the neural response of IHCs to stimuli. Figure 5 shows the DPOAEs collected before, during, and after KA injection. Results show that the fine structures of odd order DPOAE amplitudes remained essentially unchanged before the injection began and after it was completed (Figure 5, Panel A), as well as remaining stable throughout the injection (Figure 5, Panel B). This is consistent with findings from Peppi et al. (2012), where DPOAE measurements taken during an injection of KA remained stable while the CAP responses ablated. The even order measurements taken during injection were quite variable, ranging from -7 to 7 dB SPL over time. Much of this variation is the result of changes in operating point that affect even order distortions much more than odd order distortions (Lichtenhan, Brown, McLean, Chertoff, & Salt, 2011; Weiss & Leong, 1985).

DISCUSSION

CAPs have long been used to evaluate the spatial origins of neural responses to compare them to ANF tuning curves. Traditionally, derived band masking techniques and single nerve fiber recordings have corroborated CAP responses to cochlear origin. In this study, we used the novel method of progressive abolition of neural responses via ototoxic solutions to provide novel insight into the spatial origins of CAPs.

Cochlear Mechanics Are Not Impaired by Controlled Apical Injections

Varying the rate of KA injections in the cochlea allows a constant flow of pharmaceuticals throughout the cochlea, around 0.5 mm/min. This flow not only maintains a constant pressure in the cochlea (Böhmer, 1993; Salt & DeMott, 1998; Salt et al., 2015), but it allows mechanical structures in the cochlea to continue functioning normally. The DPOAEs collected before, during, and after the injection of KA show that no significant changes exist in cochlear mechanics from KA and the novel apical injection technique. There is some variability in the even order OAE's that is likely due to operating point changes (Lichtenhan et al., 2011).

Level Series and CAP Responses

When comparing 2 kHz CAP responses at various intensities to Eggermont's findings (1976), similarities are noted. The activation area of a 70 dB SL toneburst is large in Eggermont's study (Figure 1) indicating a wider area of activation by the toneburst. Similarly, the 74 dB SPL toneburst in the current study (Figure 3) was the last

to abolish, implying the stimulation area for the tone was much larger than stimulation areas for softer tones. When comparing abolition across animals (Figure 4), the 70-73 dB SPL 8 kHz tone begins to abolish 8-15 minutes after the injection begins and ends 30-38 minutes after the injection started. However, in Eggermont's study, the spread of excitation only occurred basally. In the present study, excitation occurred both basally (Figure 4, Panels B and D) and apically (Figure 4, Panel A). This new finding demonstrates variability in cochlear place maps.

The variation in abolition time noted for high intensity CAP measures is indicative of irregular spatial origins, which vary across animals. These irregularities suggest the cochlear-frequency place map is not an exponential function, as others have suggested (Greenwood, 1990; Tsuji & Liberman, 1997). Instead, it may be more like a staircase with smaller, irregular bands creating frequency place (Shera, 2015).

In his 2015 study, Shera evaluated cochlear place using a model based on basilar membrane measurements and stimulus-frequency otoacoustic emissions. When he modeled basilar membrane mobility with known irregularities in movement, it was found that reflection of the travelling wave caused the fine tonotopic microstructure to look like stair steps indicating a wide area of activation. For models tested close to threshold, it was found the steps were flatter, correlating with a more specific area of excitation on the basilar membrane. The results of the present study corroborate this finding by showing less spread of the CAP response at softer intensities.

Clinical Application

Traditionally, studies involving pharmaceutical introduce the drug from the basal end of the cochlea by allowing it to come in contact with the round window (e.g. Mikulec et al., 2008). When applying a drug to the round window, the concentration that reaches the apical portion is significantly lower than that at the base (Salt & Plontke, 2009).

Injecting a pharmaceutical apically grants more control of the concentration reaching an affected portion of the cochlea or spreading equally throughout the entire organ. This is demonstrated through the ablation of CAP responses in the present study. The apical injection approach, therefore, may be beneficial in determining the best place of application for drug therapies to the cochlea.

Additionally, this study provides the basis to determine the spatial origins of various OAE responses. Since OAEs are one of the newest diagnostic tools available to audiologists, much is still unknown about certain types of OAEs like reflection source otoacoustic emissions. Using a similar procedure to the present study, the origins of various emissions on the cochlear partition can be evaluated. This information will assist audiologists in adding additional types of OAEs to their arsenal of procedures and expand our knowledge of the physiology of the cochlea.

CONCLUSION

CAP responses are often compared to other auditory responses in order to determine the origin of those responses. The current study aimed to determine the origin of CAPs using a novel method of apical ototoxic pharmaceutical injections to inhibit neural responses. Since most pharmaceuticals are placed on the round window, injecting them from the apex allows more control of the gradation of pharmaceutical within the cochlea as it travels towards the cochlear aqueduct. CAP measures at various intensities showed irregular spatial origins, suggesting irregularity in frequency bands between animals. Additionally, DPOAEs were not affected by apical injections, indicating this novel approach does not disturb cochlear mechanics. Evaluating CAP origins utilizing apical injections will allow future studies to evaluate OAEs in the same manner, creating a comparison between CAP and OAE responses that will provide further information on the origins of OAEs to be obtained.

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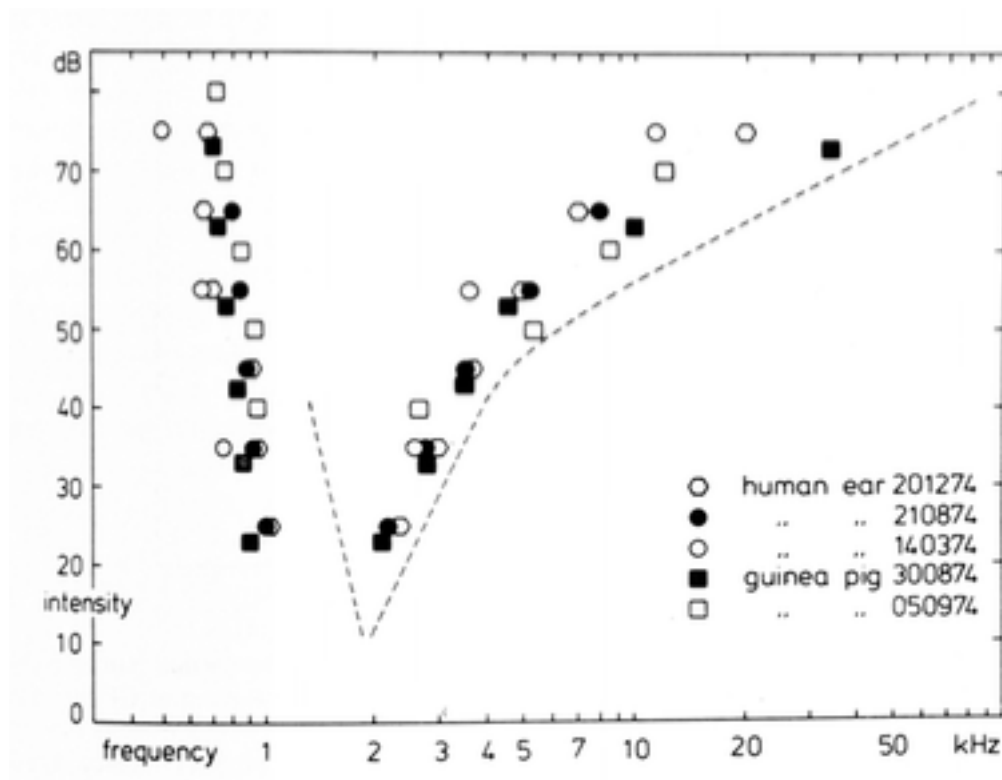


Figure 1. Response area on the basilar membrane for 2 kHz tone burst in the human and guinea pig ear. As the intensity of a sound increases, the area of excitation grows exponentially creating a shape similar to single ANF tuning curves, represented by a dotted line (from Eggermont, 1976).

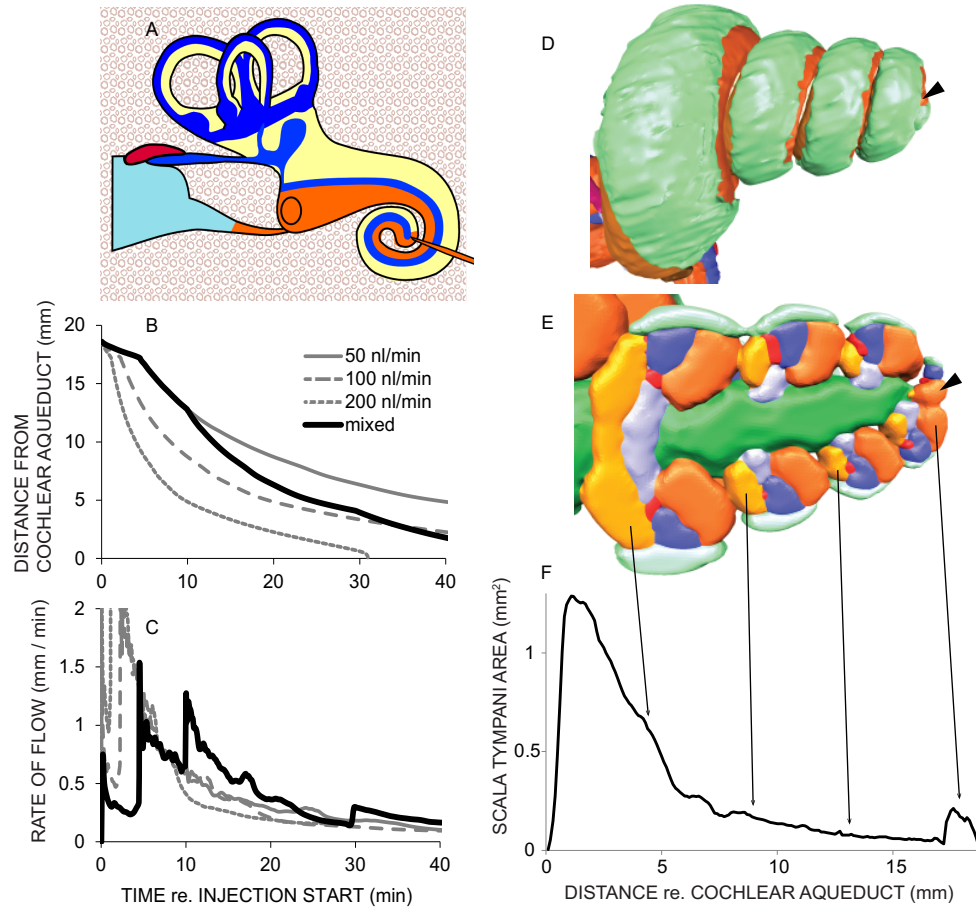


Figure 2. Left: Original apical injection procedure, basing rate of flow on measures of the cross-sectional area versus the distance along the cochlear spiral, showed the injection rate would require more variation to achieve a constant flow of 0.5 mm/min (lower left). Right: The new controlled injection varies the rate once a minute to account for changes in the cross-sectional area and allow a flow that remains more constant around 0.5 mm/min.

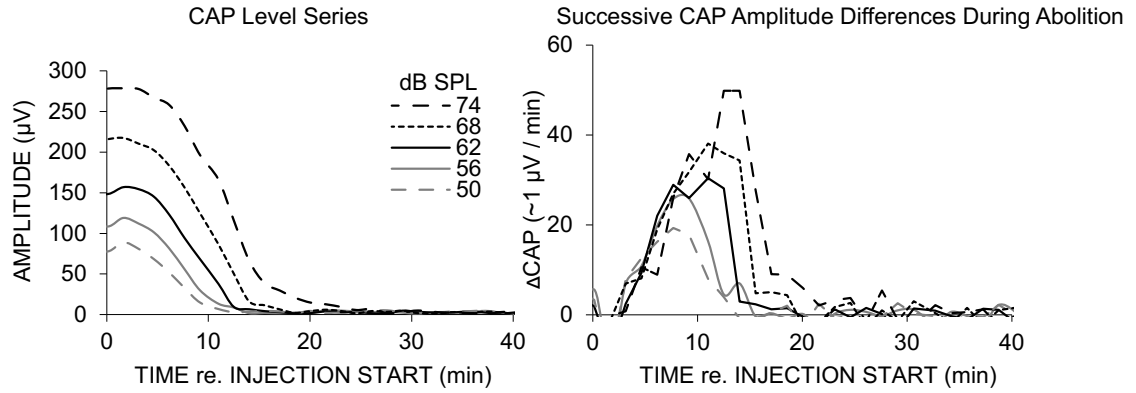


Figure 3. CAP amplitudes for a 2 kHz tone burst (left) and the difference between CAP amplitudes (right) during the injection of KA. Responses to higher stimulation levels are ablated later, indicating they originate from a more basal, high frequency region than 2 kHz.

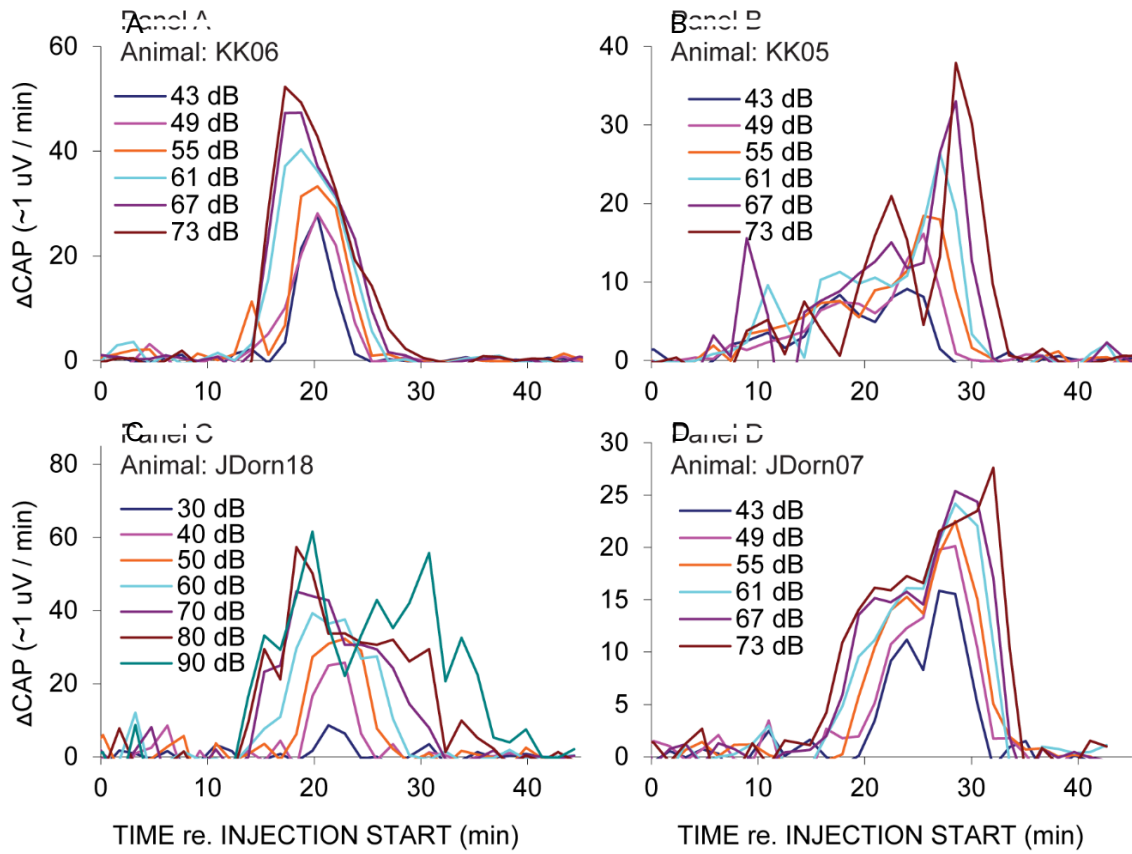


Figure 4. Difference between CAP amplitudes for an 8 kHz tone burst and various intensities during KA (Panels A-C) and TTX (Panel D) injections in four different animals. The spread of the response varies between animals. Panel A shows higher level responses peaking first. Panels B and D show higher level responses peaking last. Panel C shows peaks occurring both early and late.

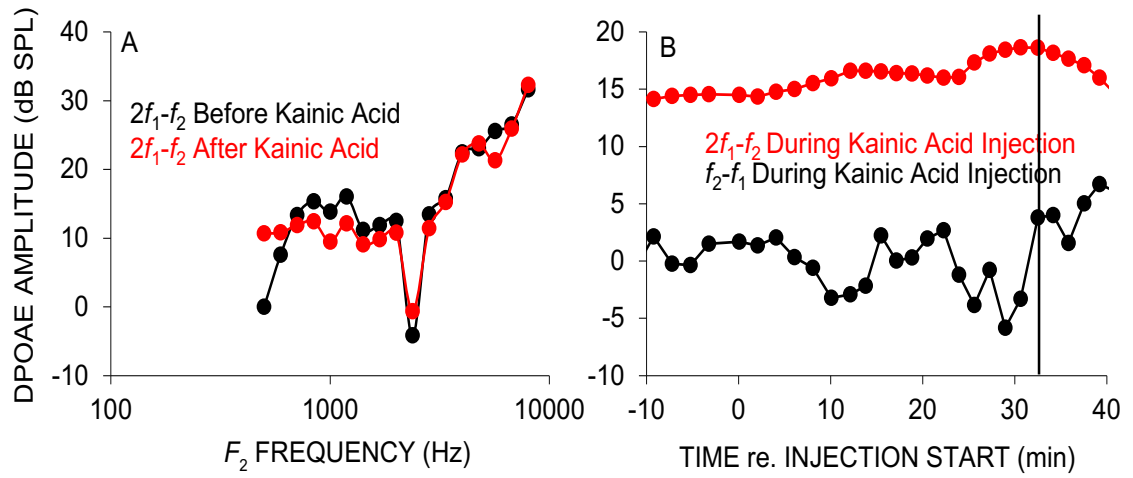


Figure 5. DPOAEs from two different experiments. Panel A shows odd order DPOAE amplitude measured before and after KA injection. Panel B shows odd and even order DPOAE amplitude measured during KA injection. The vertical line represents the point in time when the ear was fully loaded with KA at 32 minutes.