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Variation of distortion product otoacoustic emissions with furosemide injection

David M. Mills, Edwin W Rubel *

Virginia Merrill Bloedel Hearing Research Center, Department of Otolaryngology, Head & Neck Surgery, RL-30, University of Washington, Seattle, WA 98195, USA

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Abstract

Cochlear function was monitored in adult gerbils using distortion product otoacoustic emissions (DPOAE) during intraperitoneal injection of furosemide. All stimulus parameters were varied independently over a wide range, the stimulus frequencies f_1 and f_2 from 1 to 16 kHz, and the stimulus levels L_1 and L_2 from 20 to 80 dB SPL. The observed emissions at $2f_1 - f_2$ and $3f_1 - 2f_2$ could be considered to be made up of two distinct components: (1) an 'active' source which depended in a complex way on the stimulus frequencies and levels, which was dominant at low and moderate stimulus levels, and which, by definition, was eliminated by sufficient furosemide intoxication; and (2) a 'passive' source which was essentially the same at all frequencies, with a level dependence given approximately by a simple power law distribution. The change from the active to the passive source was usually accompanied by an abrupt shift in emission phase angle. A simple summation model was shown to account for the observed form of this transition. The amount of the decrease in $2f_1 - f_2$ emission amplitude after furosemide injection was approximately independent of frequency and consistent for the middle frequency ratios and intensity levels ($f_2/f_1 \cong 1.3$, $L_1 \times L_2 \cong 55 \times 50$ dB SPL). It was concluded that the combination of DPOAE with furosemide injection can usefully be employed as a probe of active cochlear mechanics.

Key words: Distortion product otoacoustic emissions; Furosemide; Active cochlear mechanics; Endocochlear potential; Gerbil

1. Introduction

Systemic injection of furosemide can be a useful manipulation for investigating cochlear auditory function (e.g., Anderson and Kemp, 1979; Evans and Klinke, 1982; Forge and Brown, 1982; Hubbard and Mountain, 1990; Ruggero and Rich, 1991; Sewell, 1984). Furosemide intoxication produces a rapid, reversible lowering of the endocochlear potential (EP) in mammals (e.g., Kusakari et al., 1978). The major functional effect noted is a reduction of the sharp peak of the basilar membrane (BM) motion at low stimulus amplitudes (Ruggero and Rich, 1991). It is believed that this reduction is caused by the decrease in driving voltage across the outer hair cell (OHC) transduction channels due to the EP decrease. This results in a decrease in the OHC function which is intrinsic to amplification of low level signals on the BM (Ashmore, 1987; review: Dallos, 1992). Thus, systemic injection of furosemide reversibly interrupts 'active' cochlear mechanics. Distortion product otoacoustic emissions (DPOAE) can provide a non-invasive method of monitoring cochlear function (review: Probst et al., 1991). Therefore, the combination of DPOAE analysis with furosemide injection (Kemp and Brown, 1984) can potentially provide a very useful assay of active cochlear mechanics.

Our initial studies (Mills et al., 1993a,b; Rubsamen et al., 1994) established that specific emissions at low stimulus levels were indeed vulnerable to an EP decrease, and were highly correlated with auditory thresholds recorded simultaneously in the anteroventral cochlear nucleus. While emissions for a wide range of stimulus intensities were monitored, only one frequency combination was employed. The present study extends this work to cover a wide range of stimulus frequencies and frequency ratios.

One primary goal of this study was to further delineate the generation of DPOAE as a function of stimu-

* Corresponding author. Fax: (206) 543-5152.

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lus parameters. It has been suspected for some time that there were two different sources of DPOAE, each evident under different conditions, sometimes denoted 'active' and 'passive' sources (Brown, 1987; Brown et al., 1989; Johnstone et al., 1990; Lonsbury-Martin et al., 1987; Norton and Rubel, 1990; Norton et al., 1991; Whitehead et al., 1990, 1992a,b). In our initial study (Mills et al., 1993a), we occasionally observed abrupt shifts in the phase of the vulnerable emissions near maximum DPOAE amplitude decrease (see also Whitehead et al., 1992b). Such abrupt shifts may be due to the uncovering of an underlying component which has a different intrinsic phase. We therefore desired to study these shifts and related phenomena in more detail. That is, our goal was to establish the relationships of various possible components, and their relative vulnerability to EP change, as a function of stimulus parameters.

An additional goal was the investigation of the behavior of active processes as a function of frequency, or place. Vulnerable DPOAE are generally believed to be generated at or very near the 'place' corresponding to the higher stimulus frequency, that is, near the location on the BM which would have a peak for a single, low intensity tone of that frequency (Kim et al., 1980; Martin et al., 1987). DPOAE therefore have some advantages over other methods, such as direct basilar membrane measurements or eighth nerve studies, in that a wide range of frequencies can be monitored continuously and non-invasively. The interruption of the active process caused by furosemide injection offers a unique opportunity to observe the effect of a decrease of EP more or less simultaneously all along the BM. A related goal was therefore to establish a baseline of the DPOAE response in the normal adult animal as a function of frequency, against which developmental changes or the effects of trauma could be adequately compared.

A potential difficulty with DPOAE measurements is that a variation of parameters can cause a change in the process of DPOAE generation and transmission (e.g., Allen and Fahey, 1993; Brown and Williams, 1993). The resulting change in emission can be difficult to differentiate from an actual change in intrinsic BM motion, i.e., a change in peak sharpness associated with a single tone input. This study was therefore designed to provide guidance as to the parameter choices likely to give the most reliable information on the functioning of the active process itself. In the present experiments, intraperitoneal (i.p.) injection of furosemide was used because of ease of administration and because it results in a slower EP change than intravenous administration (Mills et al., 1993a). This allowed us to simultaneously monitor a large number of parameters as the EP was reduced and as it recovered.

2. Materials and methods

2.1. Animal preparation

Adult gerbils (*Meriones unguiculates*) weighing 50–80 g were obtained either from a commercial supplier (Tumblebrook Farms, Brookfield, MA) or from breeding pairs in our colony originally obtained from the same source. All animal preparation and recording was performed in an I.A.C. double walled acoustic booth, maintained at 31–33°C. Animals were initially anesthetized with an i.p. injection of a mixture of ketamine hydrochloride (Ketaset; 15 mg/kg) and xylazine (Rompun; 5 mg/kg). A surgical depth of anesthesia was maintained by subcutaneous injections of the mixture (usually at 1/3 initial dosage) as needed. The pinna, surrounding skin and outer portion of the external ear canal were removed unilaterally. Using an operating microscope, the external ear canal was checked for debris and the tympanic membrane was inspected to insure that it was intact and clear. The bulla above the ear canal was exposed, and a 1 mm hole drilled in it. The tip of a small diameter, long tube (0.6 mm i.d. × 18 cm) was force-fitted into the hole to provide for equalization of static pressure between the middle and outer ears. The animal was inserted into a custom head holder, and a thermocouple was inserted in the rectum; the core temperature was maintained at 35–37°C. A microphone assembly (Etymotics ER-10B) was coupled to the ear canal with a short rubber tube (3 mm i.d. × 7 mm).

2.2. System calibration, stimulus generation and emission recording

The computer system for generating the two probe tones and detecting emissions was the same as that described previously (Mills et al., 1993a). Briefly, the system employed synchronous detection of the output of an Etymotic ER-10B microphone, resulting in a noise floor of about −20 dB SPL at a typical averaging time of 1 s. For the present experiments, the two tones generated by the computer (frequencies f_1 and f_2) were sent to two Beyer DT-48 headphones fitted to custom reducers. Each signal was delivered to the ear canal through a small tube, which went completely through the Etymotic ER-10B microphone assembly into the rubber tube coupler, ending about 3 mm from the opening to the ear canal. Stimulus signal levels of a minimum of 80 dB SPL were obtained up to 18 kHz. Closed cavity tests showed detectable instrumental distortion was at least 75 dB below the stimulus levels.

An in situ microphone calibration system was added for the present experiments. When the Etymotic ER-10B microphone was first coupled to the ear canal (and at intervals thereafter as required), a probe tube micro-

phone (Etymotic ER-7C) was connected temporarily to one of the small tubes leading through the ER-10B microphone assembly, in place of one of the transducers. This tube was the one for which the ER-7C had been calibrated by the manufacturer. The output of the ER-7C was fed to the second analog-digital input to the computer. A wide band noise signal was delivered to the ear canal through the other small tube by the remaining transducer and both microphone outputs were fast Fourier transformed (FFT). A microphone correction table was generated on the computer to correct the amplitude output of the ER-10B according to the output of the ER-7C. The ER-10B microphone output was then corrected in real time after each FFT by use of this table. This gave an absolute amplitude measurement estimated to be correct to ± 1 dB for frequencies up to 18 kHz, as measured at the end of the probe tube (i.e., 3 mm from the ear canal).

It was not possible to similarly calibrate the ER-10B phase angle in situ, however, and the phase angle correction for the microphone correction table was instead established by comparing the ER-10B response to a 1/4″ microphone (Buel & Kjer 2209) in a free-field test. While absolute phase measurements are therefore not expected to be as accurate as the absolute amplitude determinations, *changes* in phase angle with furosemide injection attributable to instrumental effects at any given pair of stimulus frequencies were generally found to be less than 10 degrees.

For every measurement, averaging for the FFT was begun after a delay of 50 ms, to avoid startup transients. For each parameter choice, the microphone output was first averaged for 0.1 s. If the measured amplitudes of the primaries deviated more than one dB from the programmed values, the attenuator settings were corrected. The microphone signal was then averaged for a programmed length of time, usually 1 s. Amplitude and phase of the FFT component at the two stimulus frequencies and at the following distortion product frequencies were recorded for each stimulus presentation: $2f_1 - f_2$, the third order term usually known as the cubic distortion tone (CDT); $3f_1 - 2f_2$, the fifth order term having, like the CDT, an emission frequency below the two primaries; $2f_2 - f_1$, the third order term with an emission frequency above both primaries; and $f_2 - f_1$, the simple difference tone.

2.3. Procedures

As soon as the animal's core temperature was stabilized, baseline readings were taken for the CDT. Usually, the parameters chosen for the baseline observations were $f_2 = 8$ kHz, $f_2/f_1 = 1.28$, and stimulus amplitudes $L_1 = L_2$ stepped from 40 to 80 dB SPL. It was required that CDT emissions be of normal amplitude and constant within 1–2 dB for 15 min before subsequent measurements began. Of ten animals, only one animal was eliminated from the study. Its CDT amplitude continually fluctuated, and we subsequently found a small amount of fluid in the bulla equalization tube. After the required baseline measurements had been made, a sequence of desired stimulus parameters was started. Typically the sequence consisted of 50–100 independent stimulus combinations which varied in f_1 and f_2 frequencies, with stimulus amplitudes L_1 and L_2 in the range 20 to 80 dB SPL. For this report, the sequence always included a 'reference' parameter setting with $f_2 = 8$ kHz, $f_2/f_1 = 1.28$, and $L_1 \times L_2 = 55 \times 50$ dB SPL. The sequence typically took a total time of 2 to 3 min to run, and was repeated automatically. After this parameter set had run for 10 to 15 min, a single intraperitoneal (i.p.) injection of furosemide (Abbott Labs) was made, at a dosage of 75 to 100 mg/kg. The same parameter sequence was then continuously monitored during the decrease of emissions and until there was a (nearly) complete recovery of emissions. A second furosemide injection was then sometimes given, or the animal was sacrificed by an injection of KCl into cardiac muscle. In either case, the parameter sequence was followed for at least another hour.

Control experiments, with saline substituted for furosemide, showed that the measurement of normal emissions was quite stable with time under the conditions of these experiments. For typical stimulus parameters, the CDT amplitude varied less than 2 dB and the phase less than 10 degrees for several hours after the saline injection. Only for parameters resulting in normal emission amplitudes less than about 10 dB SPL, or near regions containing 'notches' (where there appeared to be two components in approximate phase cancellation) did the observed variation exceed these limits.

Procedures for the care and use of the animals reported on in this study were approved by the University of Washington Animal Care Committee (re: grant NIH DC 00395, Ontogeny of sensory processes).

3. Results

3.1. Normal emissions

It is most useful to begin by establishing the normal emission characteristics over the entire parameter space: first, to further examine the claim that these emissions appear to be composed of two distinct sources; second, to obtain an adequate background to compare the changes in emissions caused by furosemide injection; and third, as a guide to choosing the much more limited set of parameters which can be followed after any one furosemide injection.

A potential difficulty in representing the results is immediately obvious. Because DPOAE require two different input tones, there are a total of four independent parameters which can be varied, the two frequencies f_1 and f_2 and two amplitudes L_1 and L_2. To represent the normal response at each emission frequency requires a five-dimensional space. One possible approach to this rather difficult visualization problem is shown in Fig. 1. The basic unit of analysis is chosen to be a contour map of emission amplitudes, determined by fixing both input frequencies and varying the input intensities independently over a two-dimensional grid of values. With a grid spacing of 5 dB for these maps, each map represents 143 measurements.

The contour map with fixed f_1 and f_2 frequencies (as seen in the individual panels of Fig. 1) was chosen

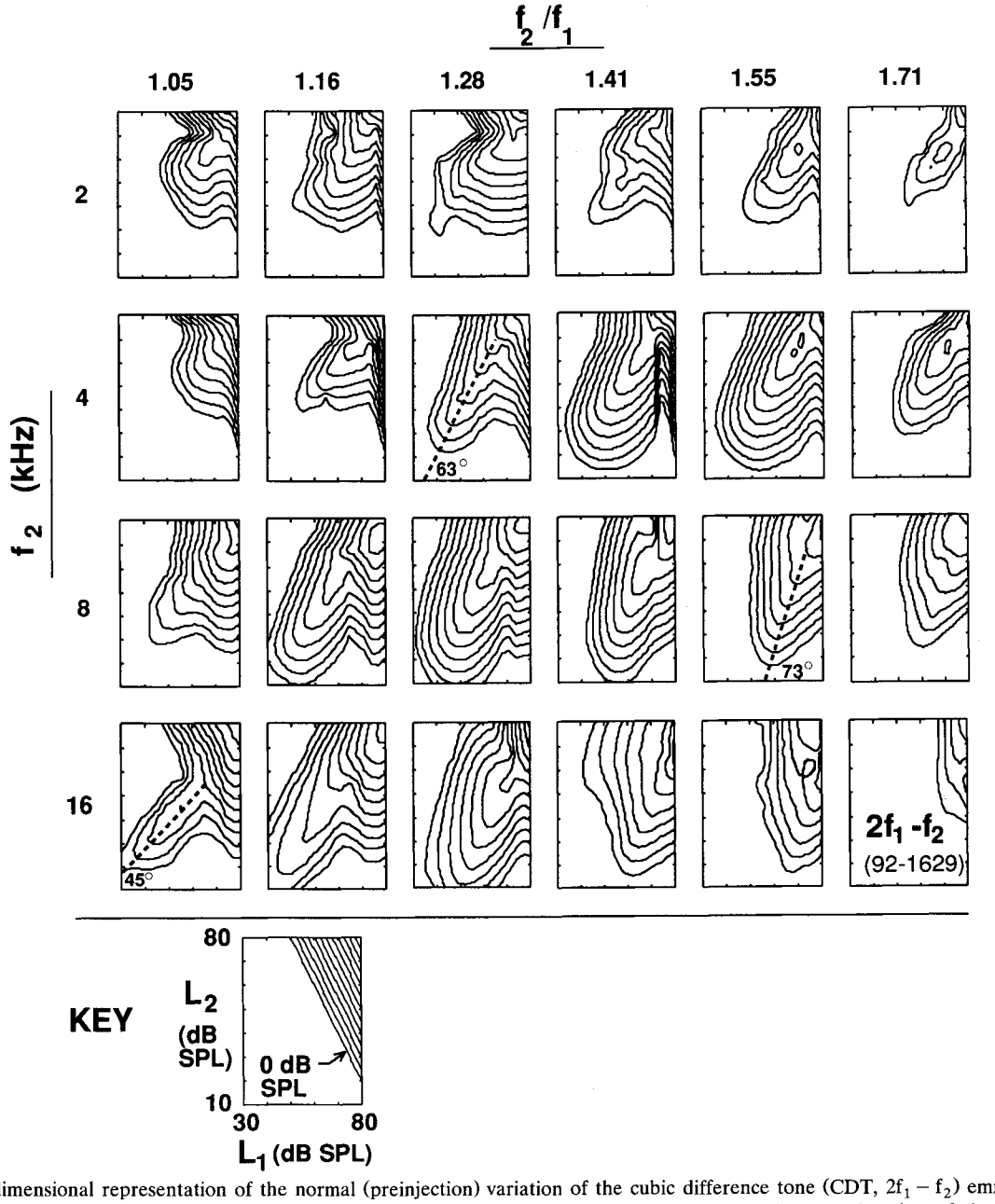

Fig. 1. A five-dimensional representation of the normal (preinjection) variation of the cubic difference tone (CDT, $2f_1 - f_2$) emission with all significant parameters. Each individual contour map presents the contours of equal emission intensity as a function of the two stimulus amplitudes, L_1 on the horizontal axis from 30 to 80 dB SPL, and L_2 on the vertical axis from 10 to 80 dB SPL. Emission contours start at 0 dB SPL and increase in 5 dB steps. Measurements for each contour map were made at 5 dB intervals in L_1 and L_2. The key illustrates the emissions resulting from a power law spectra, given by Eq. (1); the zero level is arbitrarily chosen to approximately match the power law component typically observed. The changes in these contour maps with the two input frequencies completes the specification; the f_2 frequency is represented vertically from 2 to 16 kHz, and the ratio f_2/f_1 horizontally. Note that all scales are logarithmic. The angle between the apparent ridge line and the L_1 axis is noted for reference in several of the individual contour maps.

as the most basic unit of analysis for several reasons. The most important property is that, because the frequencies for each map are fixed and the measuring system is linear, any changes in emission which occur with changes in L_1 and L_2 amplitudes must be due to the animal, and cannot be instrumental. At the sound levels and frequencies used, the middle ear reflexes are not likely to be stimulated (e.g., Avan et al., 1992), and the middle ear is expected to be linear. This means that changes in a given map are cochlear in origin. This display is also ideal for one of the purposes of the present experiments, i.e., to examine changes which occur with stimulus intensity. For each basic map, contours of equal CDT amplitudes are shown at fixed logarithmic intervals, starting at 0 dB SPL (in order to be sufficiently above the noise floor at all frequencies). The axes for each contour map are also logarithmic, as noted in the key.

A two-dimensional grid of such contour maps (Fig. 1) completes the five-dimensional representation of the normal CDT emission amplitudes. The frequency axes for the grid of grids shown are also logarithmic, although the interval on the frequency ratio axis is smaller than the f_2 frequency interval in the figure.

For normal animals prior to furosemide injection, the results may be summarized as follows. While there were certainly gradual changes with frequency, there were no obvious abrupt changes. For many frequency combinations, there appeared to be two distinct components in the response in the basic maps. One component had approximately a 'power law' emission, i.e.,

$$\text{emission amplitude at frequency } |mf_1 \pm nf_2| \propto L_1^m L_2^n \quad (1)$$

where m and n are positive integers. Emission contours which would result from this component alone are illustrated in the key to Fig. 1. The presence of an approximate power low component is obvious, for example, in the observed contours for the lower left map, for which $f_2 = 16$ kHz, $f_2/f_1 = 1.05$. In contrast, a power low component was never observed at the highest frequency ratios examined, within the range of the stimulus amplitudes employed.

For the second component, the CDT amplitude was typically maximal along a ridge extending from the region where both stimulus amplitudes were large to the region where both were small. This ridge resulted because the emission intensity was relatively reduced in regions where either of the stimulus amplitudes was large, but not both. The location and orientation of the ridge line (exemplified by the dashed lines in Fig. 1) varied with the stimulus frequencies. For low frequencies or at low frequency ratios (the top row and left column of Fig. 1) the ridge line was approximately at 45 degrees. The relative maximum in emissions occurred for $L_1 \cong L_2$, or for $L_1 \cong C\ L_2$, where C is a constant. For example, the pronounced ridge in the lower left map of Fig. 1 (for $f_2 = 16$ kHz, $f_2/f_1 = 1.05$) occurred for $L_1 \cong 4\ L_2$ (L_1 12 dB above L_2). As the frequency ratio increased, the ridge generally became more prominent and at a larger angle. For example, the ridge line noted for the case $f_2 = 4$ kHz, $f_2/f_1 = 1.28$ was at an angle of 63 degrees. At higher frequency ratios and higher frequencies (lower right quadrant of Fig. 1) the ridge line became nearly vertical, so that the relative maximum for these parameters occurred in the

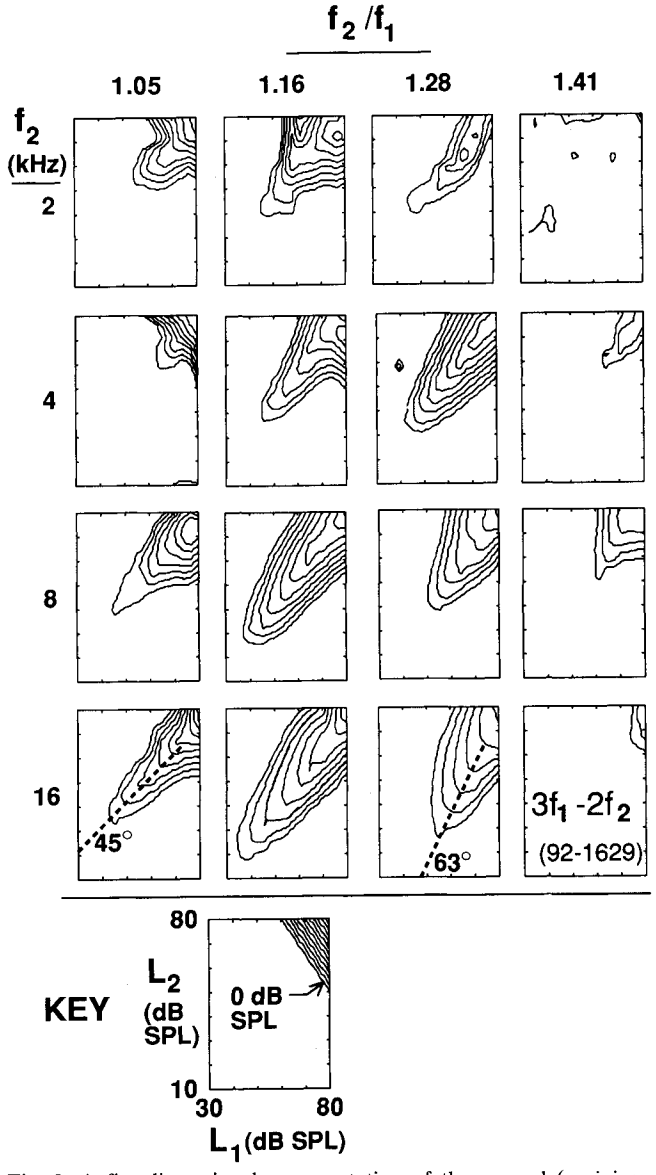

Fig. 2. A five-dimensional representation of the normal (preinjection) variation of the fifth order emission ($3f_1 - 2f_2$) with all significant parameters; same axes as Fig. 1. The key illustrates the emissions resulting from a power law spectra for this emission frequency, given by Eq. (1); the zero level is arbitrarily chosen to approximately match the power law component typically observed. Same animal as Fig. 1.

area where $L_1 = 60$ to 70 dB SPL, and was only weakly dependent on L_2.

In regions where these two components appeared to meet, there could be regions of rapid amplitude change, with the appearance of 'notches' or 'crevasses'. Examples of such notches can be observed on the upper left of contour maps at low frequencies and low frequency ratios (upper left maps in Fig. 1) and in the lower right of maps for higher frequencies and middle frequency ratios (e.g., middle maps in Fig. 1). The phase of the emission was typically found to change rapidly across these notches (data not shown). Such notches are not always seen; it is obvious from these two dimensional maps that the two components could also merge smoothly for many frequency and frequency ratio combinations.

A similar presentation for the fifth order emission ($3f_1 - 2f_2$) appears in Fig. 2. There are several obvious differences between this frequency and the CDT. The fifth order emission was not detectable at higher frequency ratios (e.g., Brown and Gaskill, 1990). While the fifth order term also appeared to consist of two components, the power law component was only obvious at the lower frequencies and frequency ratios (upper left area in Fig. 2). The contours for the ridge component had a narrower appearance than the CDT; the difference can be related to the fact that the rate of increase of emission coming up the face of the ridge (from the lower left in each map) was smaller for the firth order term. The angle of the ridge line, however, showed the same trend as did that for the CDT, starting at about 45 degrees for lower frequency ratios, and appearing to steepen as the ratio increased. Representative angles for the ridge lines are noted by dashed lines in individual contour maps.

3.2. *Changes in emissions with furosemide injection: overview*

Ideally, one would like to follow the changes with furosemide injection for the whole parameter space. However, more than 2 h are required to obtain the data for a complete representation such as Fig. 1. Even with i.p. injection, the rate of change induced by furosemide requires monitoring a given parameter choice at least every 1–5 min, depending on the time resolution desired. Obviously, only a very reduced set of parameters can be followed with any one animal. A total of nine animals was used to cover the parameter choices included in this report.

The first set of parameters chosen allowed the determination of changes in time of three contour maps,

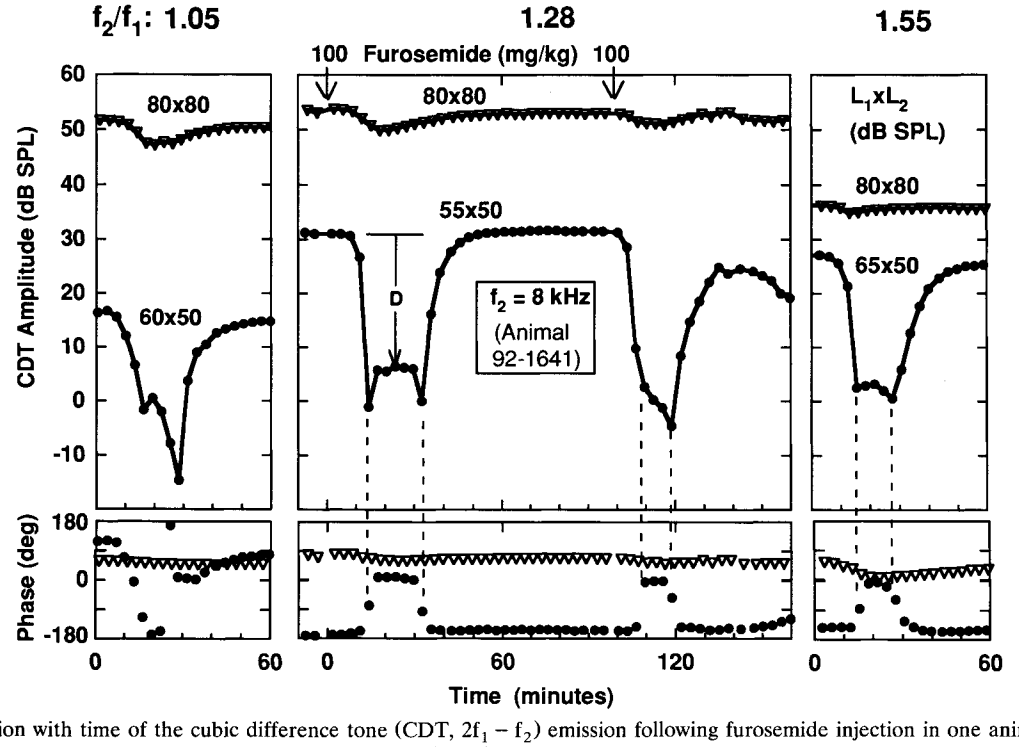

Fig. 3. The variation with time of the cubic difference tone (CDT, $2f_1 - f_2$) emission following furosemide injection in one animal, for stimulus frequency $f_2 = 8$ kHz, and for three different frequency ratios (f_2/f_1). Emission amplitudes are shown in the upper plots, with corresponding phase angles below; the dashed vertical lines simply connect corresponding amplitude and phase measurements taken at the same time. The phase angle zero reference is arbitrary at each frequency ratio. There were two 100 mg/kg furosemide injections at the times shown by the arrows at the top of the middle panel (at $t = 0$ and $t = 99$ min). Parameters listed are the stimulus amplitudes $L_1 \times L_2$ in dB SPL. The dimension D is defined as the decrease from the preinjection amplitude to the middle of the flat minimum, as shown. The right and left panels show changes after the first furosemide injection only.

each at a different frequency ratio, all at the same f_2 frequency. By using grids at 10 dB intervals for each basic contour map, three complete contour maps could be generated every 3 min. Before considering the changes in entire contour maps, however, we will consider the variation with time of emission amplitudes at several discrete parameter choices, shown in Figs. 3 and 4. At each frequency ratio, the variation with time of the CDT emission is presented for two different stimulus amplitude pairs. One has both stimulus amplitudes equal to 80 dB SPL. The other has $L_2 = 50$ dB SPL, with L_1 chosen using the normal contour maps (Fig. 1) so that the CDT emission was approximately on the preinjection ridge. This was done so that the levels would be as equivalent as possible for the same f_2 frequency given the changes in frequency ratio. Fig. 3 presents changes seen with $f_2 = 8$ kHz. For comparison, the emissions with $f_2 = 2$ kHz are presented in Fig. 4 for approximately the same parameter choices as in Fig. 3.

The results may be summarized as follows: For all frequencies, there was very little change in emission amplitude at the highest level monitored, but at the lower stimulus levels there was a sharp decrease in CDT emission amplitude following furosemide injection. The behavior following the first injection for $f_2/f_1 = 1.28$ in Fig. 3 is particularly noteworthy: there was a very sharp minimum, a partial rebound to a flat, relatively unchanging response, another sharp minimum, followed by a rapid return to preinjection levels. The change in phase angle was even more rapid than the change in amplitude; note that the phase angle changed significantly only at and between the two sharp minima (see the dashed vertical lines in Figs. 3 and 4). Such obvious sharp minima were not always observed (they simply could have been missed with the typical sampling rate being only once every 3 min). The other minima in Figs. 3 and 4 were therefore like those more typically observed. The amplitude for these cases also dropped sharply to a more or less flat minimum, and most of the change in phase angle occurred during the transitions between the rapidly varying component and the flat minimum. This behavior was usually more obvious and consistent for frequency ratios above 1.05, as will be discussed later.

In general, the direction of the phase change was an increase of angle (a decrease in phase lag) of about 180 degrees. Usually, the result of the change for the emissions at low stimulus amplitudes was that the emission angle during the flat part of the minimum changed to become very nearly the same as the emission angle at high stimulus intensities. Note, however, that typically after furosemide injection the low level angle in effect rotates *away from* the high level angle to end up closer to it at maximum effect.

The center graphs of Figs. 3 and 4 illustrate the

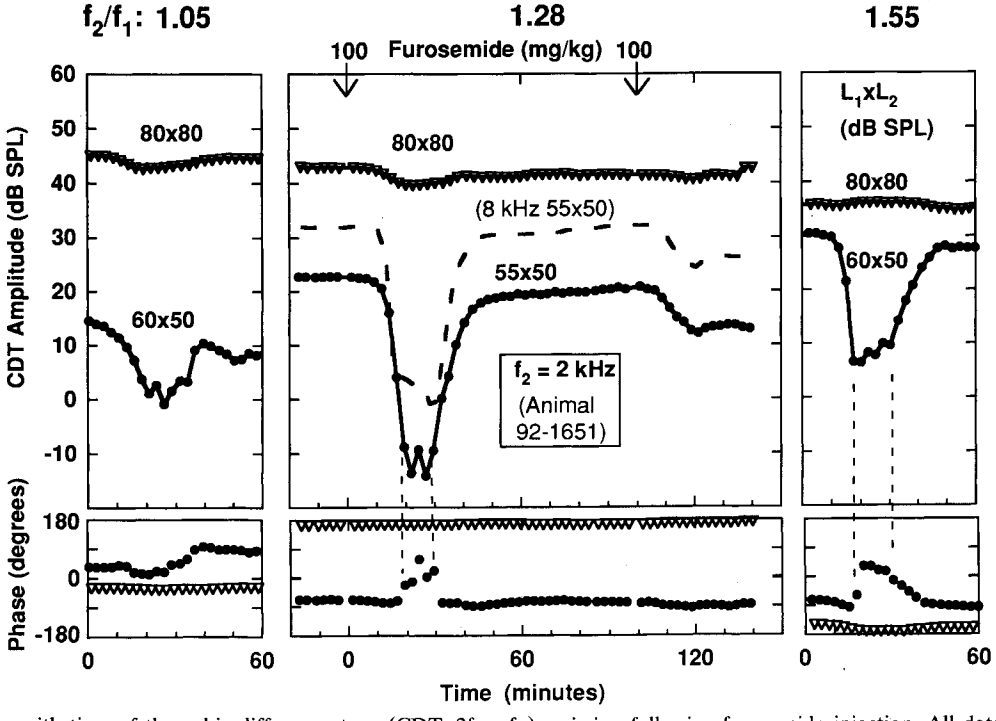

Fig. 4. The variation with time of the cubic difference tone (CDT, $2f_1 - f_2$) emission following furosemide injection. All data are for stimulus frequency $f_2 = 2$ kHz, except for the dashed line in the center panel which shows the CDT amplitude for the reference parameter set ($f_2 = 8$ kHz, $L_1 \times L_2 = 55 \times 50$ dB SPL). Emission amplitudes are shown in the upper plots, with corresponding phase angles below. The phase zero reference is arbitrary at each frequency ratio. There were two furosemide injections at the times shown. Parameters listed are the stimulus amplitudes $L_1 \times L_2$ in dB SPL.

effect of a second furosemide injection, which was sometimes given after there was full recovery of emissions following the first injection. Often the second injection had less effect than the first. However, if there was a sharp decrease to a flat minimum, the level of the minimum reached was usually the same as, or slightly less than, the first. There usually was not a full recovery after the second injection. For those animals which were given KCl into cardiac muscle instead for the second injection, the vulnerable emissions usually decreased sharply to a flat plateau, exactly as for a second furosemide injection. After holding this level for a few min, these emissions then decreased to the noise floor and did not recover.

For the animal in Fig. 3, the decrease in amplitude of the CDT from preinjection level to the flat minimum (D) was 26 dB. This amount of decrease was a very typical value: it ranged from 23 to 31 dB for the first injection in the nine different animals comprising the experimental group, for the reference level stimulus parameters ($f_2 = 8$ kHz, $f_2/f_1 = 1.28$, $L_1 \times L_2 = 55 \times 50$ dB SPL). The associated phase shift was always positive (i.e., the phase lag decreased) and between +160 and +190 degrees, excluding one animal for which the emission amplitude did not decrease to a flat minimum after the first injection. When there was a second injection which caused the emissions again to reach a flat minimum, the phase shift at the second injection was similar to the first. An example is seen following the second injection in Fig. 3.

Fig. 4 illustrates corresponding changes for a different animal for a frequency $f_2 = 2$ kHz. The CDT emission amplitude at the reference parameter set ($f_2 = 8$ kHz, $f_2/f_1 = 1.28$, $L_1 \times L_2 = 55 \times 50$ dB SPL) for this same animal is shown by the dashed line. It is apparent that, even though the normal emission contours for the two frequencies appeared to be quite different (Fig. 1), the responses to furosemide injection at the same frequency ratio were very similar. In other words, the response to furosemide injection varied more with the stimulus frequency *ratio* than it did with the stimulus frequencies themselves. For this animal the decrease in CDT emission at the reference parameter set was about 29 dB. While the normal CDT amplitude at 2

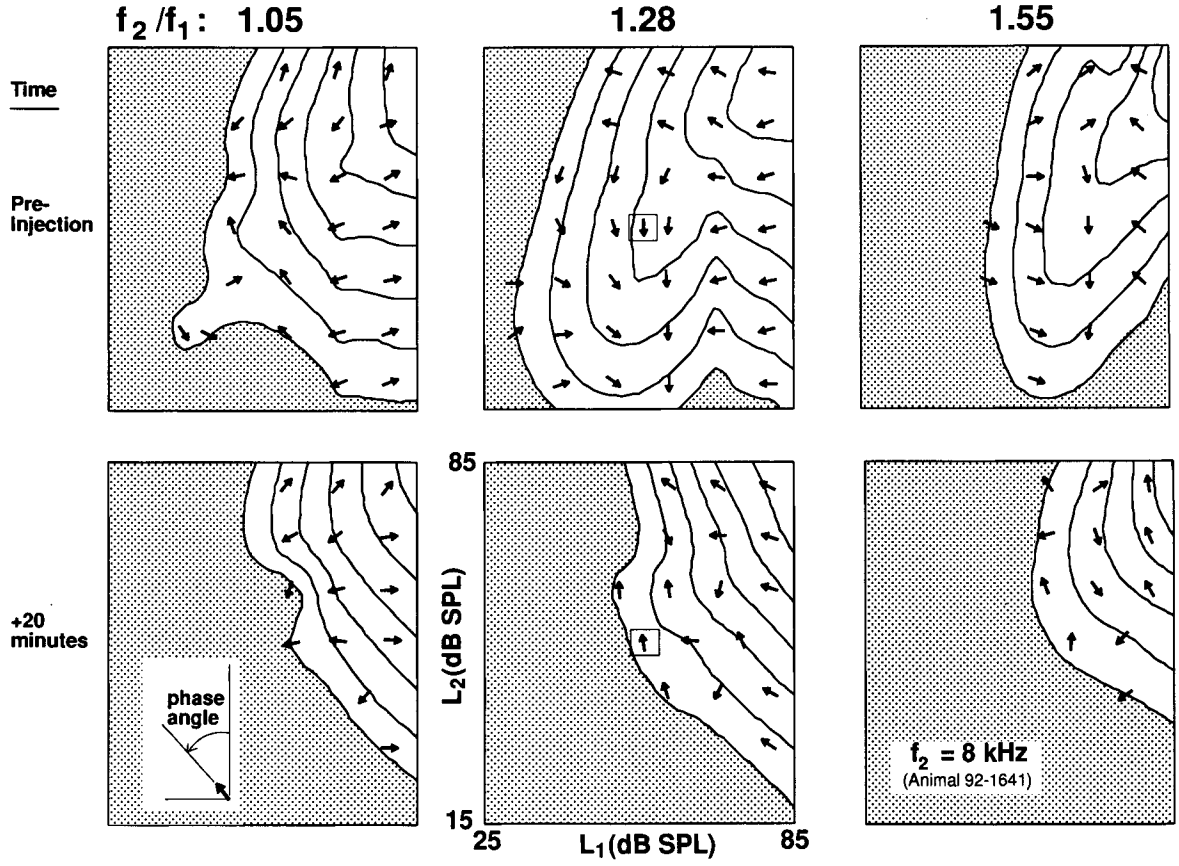

Fig. 5. The change in the contour map of the cubic distortion tone (CDT, $2f_1 - f_2$) emission from preinjection (top panels) compared to the time at the middle of the flat minimum, 20 min postinjection (bottom panels), for $f_2 = 8$ kHz. Contours of emission amplitude are shown as a function of the stimulus amplitudes, L_1 and L_2. Emission amplitudes are shown from 0 dB SPL, increasing in 10 dB steps. Emission phase angles are shown at selected sites (stimulus amplitude pairs) by the arrows. Note that the zero phase reference is arbitrary for each column of maps. A relative increase in phase (a decrease in phase lag) is plotted counterclockwise, as noted in the lower left of the figure. Measurements for these contour maps were made at 10 dB intervals in L_1 and L_2. The square denotes the 55 × 50 dB SPL reference level. Same animal as Fig. 3.

kHz for the otherwise same parameters was 10 dB lower than at 8 kHz, the magnitude of the net decrease to the flat minimum was very nearly the same as that at 8 kHz.

3.3. Changes in contour maps at maximum effect

The preinjection contour maps are now compared with those obtained after the first furosemide injection. The postinjection maps shown were chosen at the time of 'maximum effect', defined as the time that the CDT amplitudes for the lower stimulus levels were in the middle of the flat minimum. Figs. 5 and 6 present these contour maps for the same two animals as Figs. 3 and 4 respectively. Both figures compare preinjection contour maps with those determined in the middle of the flat minimum, about 20 min postinjection. Note that the resolution of the preinjection maps is the same as the postinjection maps in these figures (i.e., 10 dB intervals were used) and is poorer than that used for Figs. 1 and 2 (5 dB intervals). For direct comparison, the outlines of the corresponding maps at 8 kHz are repeated in the 2 kHz maps in Fig. 6.

From Fig. 6, it is immediately obvious that there were significant differences between the preinjection maps at the two f_2 frequencies. However, there was less difference between the two maps at maximal furosemide effect. There was a similar small variation with frequency ratio at both f_2 frequencies. Again, the differences found at maximal effect appeared to depend more on frequency ratio than on frequency.

The contour maps at maximum effect could all be roughly approximated by the *same* power law distribution. However, while it appears that the contours for the passive component were approximately at the *angle* appropriate to a power law (i.e., a slope for the contour lines of 2:1) the gradient (i.e., the inverse of the distance between contours) was not usually quite as steep as a power law would be. Also, the overall amplitude of the passive component decreases slightly as the frequency ratio is increased. The result for the fifth order term ($3f_1 - 2f_2$) was essentially the same; the contour maps at maximum effect were approximately given by a power law source. However, because of the generally lower emission intensity of this term, there were insufficient data points above the noise

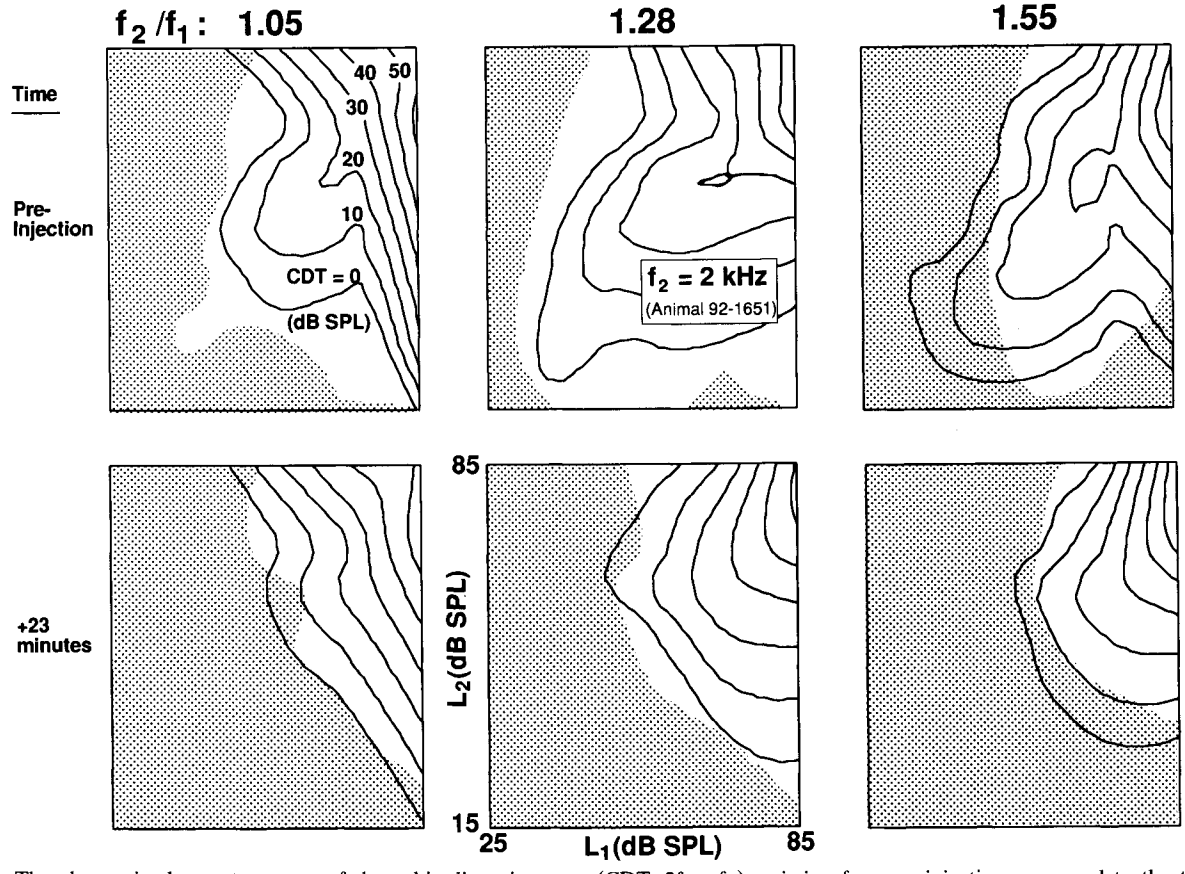

Fig. 6. The change in the contour map of the cubic distortion tone (CDT, $2f_1 - f_2$) emission from preinjection compared to the time at the middle of the flat minimum, 23 min postinjection, for $f_2 = 2$ kHz. Contours of emission amplitude are shown as a function of the stimulus amplitudes, L_1 and L_2. Emission amplitudes are shown from 0 dB SPL, increasing in 10 dB steps, as noted in the left map. For comparison, the outlines of the 0 dB SPL contours for the emission for $f_2 = 8$ kHz from Fig. 5 are shown by the shading. Measurements for these contour maps were made at 10 dB intervals in L_1 and L_2. Same animal as Fig. 4.

floor at maximum effect to derive accurate contour maps, and they are not presented.

The component remaining at maximum effect will generally be termed the 'passive' component, even though it is obvious that the contours only approximate the power law and that there are probably still contributions from active processes at the furosemide dosages employed in this study.

The component which is vulnerable to a change in EP is well delineated by the changes occurring in the maps, comparing postinjection to the maximum effect in Figs. 5 and 6. As a whole, it is clear that this 'active' component was not nearly as dominant at a frequency ratio of 1.05 as it was at higher ratios. However, the active component at the smaller ratio did dominate the passive at the center of each map, i.e., for moderate intensities. The active component at $f_2/f_1 = 1.05$ was also found to be more variable across animals than that for higher frequency ratios – compare the preinjection maps in Figs. 5 and 6 with Fig. 1, especially for $f_2 = 8$ kHz.

Both amplitude and phase information are given in Fig. 5. Note that because of difficulty in determining absolute phase information, phase differences between different frequency ratios are meaningless. However, phase changes observed at a given frequency ratio (within a contour map or between pre- and post-injection maps) are significant. First, in the map for the f_2/f_1 ratio of 1.28 there is a rapid phase change of about 90 degrees (not obvious at this resolution) just to the left of the notch located at $L_1 = 70$ dB SPL. Comparing pre- and post-injection phase information for all the maps, note that the phase angles did not change much in areas where either stimulus amplitude was large (along the top and right sides of each contour map). On the other hand, the behavior for points for low stimulus amplitudes can be illustrated with that for the reference point (the location of the open square in Figs. 5 and the 55×50 emission in Fig. 3). Pre-injection, the phase was about 180 degrees (straight down in Fig. 5.) As the emission amplitude hit the flat minimum, the phase rotated 180 degrees counterclockwise, ending up near zero degrees (pointing upward in Fig. 5).

3.4. CDT trajectories as a function of stimulus level

The trajectories of the CDT emission with stimulus amplitude as a parameter are shown in Fig. 7. For this case, a smaller furosemide injection (75 mg/kg) was chosen to avoid decreasing the emissions to the flat minimum where possible. Fig. 7a shows the variation of emissions with time for the first hour following injection. Fig. 7b plots measured emission amplitudes versus those at $L_1 \times L_2 = 55 \times 50$ dB SPL. This second plot is presented to show the correlation between emissions resulting from different stimulus levels. It includes all data for two injections over 4 h, except that points which were obviously associated with a 'flat' minimum (e.g., the points between the two sharp minima for the 45×30 trajectory in Fig. 7a) or at the noise floor were deleted for clarity.

For stimulus levels of 60 dB SPL or lower, the correlation between the amplitudes at different levels

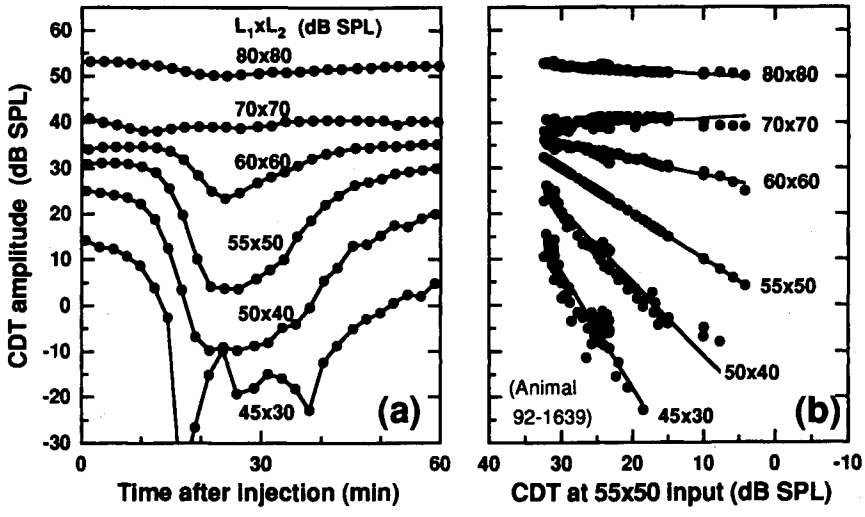

Fig. 7. The variation of cubic difference tone (CDT) amplitude after furosemide injection at different stimulus levels, all at $f_2 = 8$ kHz and $f_2/f_1 = 1.28$. There were two 75 mg/kg furosemide injections 2 h apart. (a) Variation with time for the first hour following the first injection. (b) Emission amplitudes plotted versus emissions at the 'reference level', 55×50 dB SPL. All data for four hours of recording are included in b except points obviously at a flat minimum or at the noise floor. Note that at any given time the decreases in emissions for stimulus levels below the reference level (55×50 dB SPL) are relatively greater than the emissions from the reference level, while the decreases for stimulus levels above are relatively smaller.

is very good. The emission amplitudes are obviously 'nested' for this sequence of stimulus amplitudes; the emissions for lower stimulus levels change more in proportion to those at higher levels. The time variation between stimulus levels is, however, very similar. That is, the emission amplitudes for all the stimulus levels began to decrease at about the same time and to recover at about the same time.

For interpreting these results, note that the sequence of stimulus levels has generally been chosen so that the preinjection emission lay approximately on the ridge of emission seen in Fig. 1, and areas with 'notches' were therefore generally avoided. The fact that the 70×70 level was not far from a notch (e.g., see Fig. 5) may explain its inverse correlation with the other levels. The correlation was also found to be considerably poorer between similar stimulus levels at a frequency ratio of 1.05, compared to those at the moderate ratios shown in Fig. 7, as will be seen next.

3.5. DPOAE trajectories as a function of frequency ratio

The observed variation with time of both the CDT and the fifth order ($3f_1 - 2f_2$) term are presented in Fig. 8, for 3 different frequency ratios and several different amplitudes. The amplitude parameter listed is the L_2 level; the L_1 levels were chosen equal to the L_2 levels except at the lower stimulus levels, where they were chosen so that the CDT emission was approximately at the preinjection ridge maximum (values chosen are listed in the figure legend).

While there are some obvious similarities in the responses at all frequency ratios for both distortion components, there are also some obvious trends. The CDT responses at the middle frequency ratio were generally the most stable, consistent and reproducible. In contrast, at the lower frequency ratio, the amplitude response at all levels was somewhat more variable, even though the phase response was usually quite stable. This behavior continued for the middle frequency ratio for the fifth order term and even for the CDT for the 70×70 stimulus. While the CDT at a frequency ratio of 1.55 showed the stability characteristic of that at the 1.28 ratio, the 65×50 emission at that ratio did not clearly decrease to the flat minimum as did the emission at the same L_2 level at the middle frequency ratio. At the higher ratio, the fifth order term was generally too low in intensity to measure (e.g., see Fig. 2).

Note also that Fig. 8 allows the comparison of different emission frequencies present in the same signal. For example, consider the 60 dB SPL stimulus in the center two panels ($f_2/f_1 = 1.28$). Following furosemide injection, the $2f_1 - f_2$ signal barely decreased to the passive component, while the $3f_1 - 2f_2$ signal spent a considerable time at a more or less flat minimum (there was a characteristic phase shift, not shown, observed during this minimum to support this

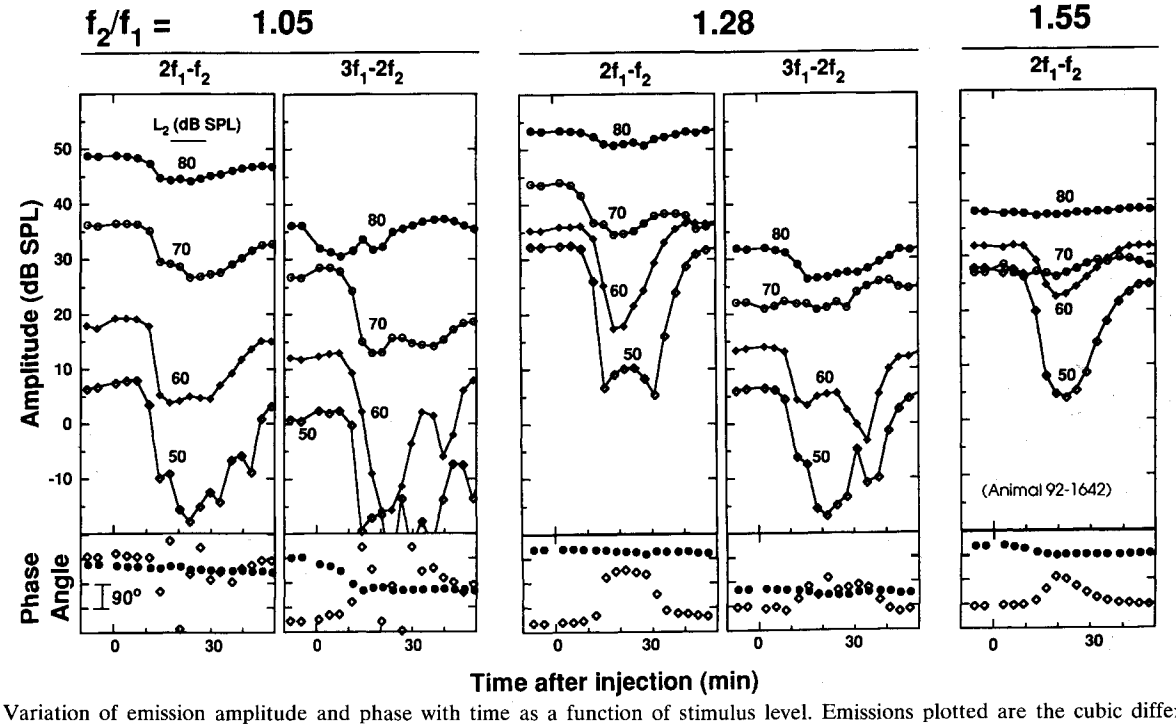

Fig. 8. Variation of emission amplitude and phase with time as a function of stimulus level. Emissions plotted are the cubic difference tone (CDT, $2f_1 - f_2$) and the fifth order term ($3f_1 - f_2$) at three different frequency ratios, all with $f_2 = 8$ kHz. The parameter listed is the L_2 stimulus amplitude; L_1 was equal except that the lower stimulus levels for $f_2/f_1 = 1.28$ were $L_1 \times L_2 = 55 \times 50$ dB SPL, and for $f_2/f_1 = 1.55$ were 70×60 and 65×50. Dosage was 100 mg/kg furosemide.

interpretation). The passive component therefore dominated one of the odd emission frequencies but not the other.

3.6. CDT trajectories as a function of f_2 frequency

Typical trajectories of CDT amplitudes with f_2 frequency as a parameter are summarized in Figs. 9 and 10. For all data presented in these two figures, $f_2/f_1 = 1.28$ and $L_1 \times L_2 = 55 \times 50$ dB SPL. Fig. 9 provides the variation of the emissions with time for f_2 at octave intervals from 1 to 16 kHz. While there are minor differences in the slope of the initial decrease and of the recovery, it is obvious that the major sharp decrease occurred essentially simultaneously across the entire frequency range. The minor differences in the onset and recovery are very reminiscent of the differences due to differences in stimulus amplitude (Fig. 7). It can be said that the lower frequency emissions (for $f_2 = 1$–4 kHz) appear as if, for the same sound levels in the ear canal, the effective stimulus at the cochlea was at a somewhat lower level than at higher frequencies.

The amount of the decrease from the preinjection value to the flat minimum (the distance *D* defined in Fig. 3) is plotted versus f_2 frequency in Fig. 10a for the same parameter values as in Fig. 9. It can be seen that the decrease was remarkably constant with frequency, especially given that the absolute values of the preinjection amplitudes for this stimulus varied considerably with frequency (e.g., see Figs. 1, 4 and 9). For the nine f_2 frequencies at half-octave intervals from 1 to 16 kHz, the observed decrease *D* was between 20 and 27 dB, with an average of 22 dB. There may have been a slight tendency for D to increase with increasing frequency.

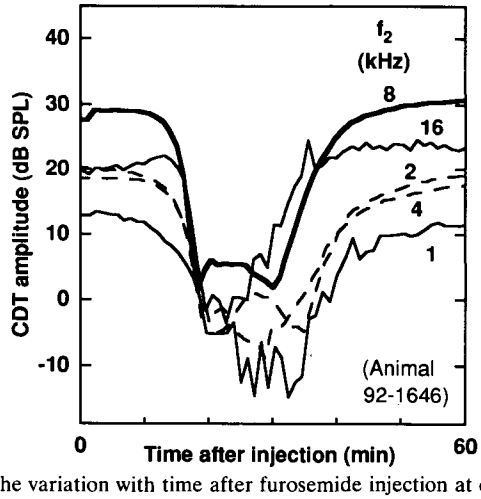

Fig. 9. The variation with time after furosemide injection at different f_2 frequencies for cubic difference tone (CDT), all at stimulus levels $L_1 \times L_2 = 55 \times 50$ dB SPL and frequency ratio $f_2/f_1 = 1.28$. Each frequency was sampled once a minute. Dosage was 100 mg/kg furosemide.

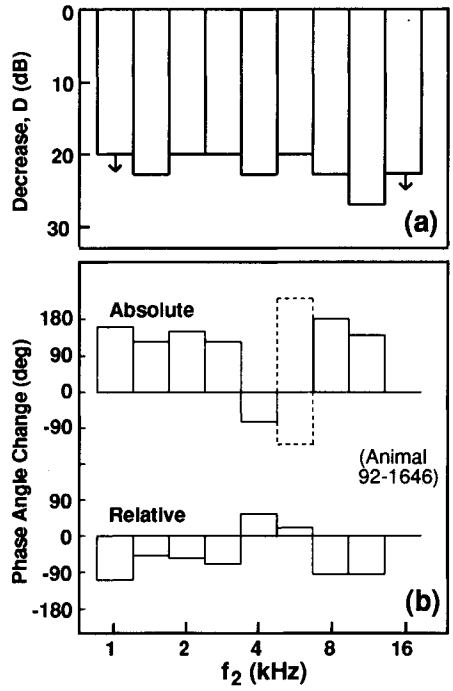

Fig. 10. The variation with f_2 frequency of the response at maximum furosemide effect. Measurements were made at half octave intervals for f_2 from 1 to 16 kHz, repeated at 1-min intervals. Other parameters were $f_2/f_1 = 1.28$ and $L_1 \times L_2 = 55 \times 50$ dB SPL. Same animal as Fig. 9. (a) The variation of the decrease, *D*, defined in Fig. 3. The arrows indicate that the minimum amplitudes at 1 and 16 kHz were uncertain due to noise contamination. (b) Change of phase angle at maximum effect versus f_2 frequency. The magnitude and direction of the absolute change – the angle at maximum effect compared to its own preinjection value – is given on the top line, an increase implies an increase in phase lead (a decrease in lag). The relative change is defined as the change in the emission phase angle resulting from the lower level stimulus *relative* to the phase angle for the corresponding $L_1 \times L_2 = 80 \times 80$ dB SPL emission. Intermediate angles are not taken into account for the relative shifts, so the maximum possible relative change is 180 degrees; a negative shift (below the line in the figure) represents a decrease in the difference in the relative phase between the two emission; that is, the two angles were closer together at maximum effect than they were originally. The phase shift at 16 kHz was not included because the signal was too noisy at maximum effect. Two possible values for the absolute phase shift are shown for the 5.6 kHz response because the direction of the shift was ambiguous: see text.

In contrast to the constancy of the magnitude of the decrease, the phase change at maximum furosemide effect did vary somewhat with f_2 frequency. For these measurements, each parameter choice was observed once every minute in an effort to reduce ambiguity at the sudden phase changes. For this animal, at all frequencies other than $f_2 = 5.6$ kHz (see below) there was an unambiguous, reversible phase shift of 180 degrees or less. The top panel of Fig. 10b shows the variation of the *absolute* phase change with f_2 frequency, i.e., the phase angle at maximum effect compared to its own initial value. Below is plotted the *relative* change, defined as the change in the angle

between the emission for the 55 × 50 stimulus and that for the 80 × 80 stimulus measured at the same time.

Except for an apparent transition near 4–5 kHz, it can be seen that the absolute phase angle of the emission generally increased about 130–180 degrees, with the result that there was generally a *decrease* in the relative angle between the emissions at stimulus levels from 55 × 50 and those from 80 × 80 dB SPL. That is, these two angles were generally closer together at maximum effect than they were normally. For example, at 1 kHz, the CDT absolute phase shift was + 160 degrees, meaning that the phase lag of the emission for the 55 × 50 stimulus pair decreased by 160 degrees at maximum effect. The corresponding relative shift was − 110 degrees, meaning that the phase angle between the emission for the 55 × 50 stimulus relative to the 80 × 80 stimulus decreased by 110 degrees.

The only exceptions to this behavior for this animal were for frequencies near 4 kHz. At $f_2 = 5.6$ kHz, for example, the observed phase shift was actually ambiguous. It could be considered a reversible increase of about 210 degrees, or a decrease of about 150 degrees, followed during recovery by another decrease of about 200 degrees. Also, at this frequency as well as at 4 kHz, the relative angle between the CDT emissions at the 55 × 50 and 80 × 80 stimuli actually increased slightly at maximum effect. However, the shifts near 4 kHz were not consistent across animals. For example, the absolute shifts at 4 kHz were zero and + 180 degrees for two other animals. In contrast, the phase shifts at frequencies away from 4–5.6 kHz were quite consistent across animals, as were the amplitude decreases, *D*, at all frequencies.

3.7. Observations of other distortion frequencies

Finally, observations of the other two distortion products recorded ($2f_2 - f_1$ and $f_2 - f_1$) were also extended to a wider range of stimulus parameters, compared to the initial observations made at one pair of stimulus frequencies (Mills et al., 1993a). The behavior noted in the initial study was found with only minor variations over the entire parameter space. The variation of the other cubic term ($2f_2 - f_1$) was again found to be similar to that of the other odd order terms, but typically lacked the sharp decrease found for the 'vulnerable' terms, $2f_1 - f_2$ and $3f_1 - 2f_2$. Depending on parameters, however, this cubic term could also show the 'bottoming out' behavior seen in the other odd order terms. As before, the difference tone ($f_2 - f_1$) variation was found to be completely unlike the odd order terms, but appeared *related* to them. It typically went through an apparent zero (a minimum in amplitude and a sharp phase transition) *near* the time when the *slope* of the CDT amplitude as a function of time was changing to pass near zero. For example, such an apparent zero was usually observed in the difference tone amplitude near the time that the CDT recovered fully, about 45 min post-injection. Neither the difference tone nor the other cubic term appear potentially as useful as the CDT and fifth order term for directly monitoring the function of the cochlear amplifier, and are not considered further here.

4. Discussion

4.1. Active and passive components in distortion product emissions

The data presented here indicate that it can be very useful to think of the emissions for both the CDT ($2f_1 - f_2$) and fifth order ($3f_1 - 2f_2$) terms as originating in two different 'components'. The 'active' component, by *definition* the component which is strongly decreased by an abrupt change in EP, normally dominates the response at low and moderate intensities at all frequency ratios. At higher intensities, and 'underlying' the active component at lower stimulus levels, is a 'passive' component. By definition, the passive component is that which is observed when the active component is maximally interrupted by furosemide intoxication. This passive component has approximately a power law distribution and is much the same at different frequency ratios and different frequencies. The main change appears to be that the intensity scale factor appears to decrease slightly with increasing frequency ratio, so that the passive component is relatively lower in intensity, and thus more difficult to observe, as the frequency ratio is increased.

In contrast, the distribution of the active component changes substantially with frequency and frequency ratio. Consider, for example, the orientation of the ridge line defined by the location of the relative maximum of the active component in Figs. 1 and 2, illustrated by the dashed lines in several of the maps. At low frequencies and/or low frequency ratios, the ridge line lies near 45 degrees, while at high frequencies *and* high ratios, the ridge line is more nearly vertical. There are a number of other obvious changes in the emission maps over the entire parameter space, including the presence or absence of notches, the relative strength of the active component, etc. Even at the same frequency ratio, there are changes in the distribution of emissions observed as the f_2 frequency is changed. (One can, in fact, notice more similarities between two maps in adjacent rows in Figs. 1 and 2 if, as the f_2 frequency is increased, the frequency ratio is decreased by about one interval. This last observation is probably related to the fact that, to get the same degree of overlap between the excitation patterns of two traveling waves having different frequencies, the frequency ratio be-

tween the two stimuli must be decreased as one moves from low to high frequencies; e.g., see derived traveling wave amplitudes in Allen and Fahey, 1993).

The proposal that there are two relatively independent components is enhanced by the observation that there is usually a sudden change in phase as the active component decreases, around the time when the emission decreases to a relatively constant response. In this study, the change in phase was most frequently (but not always) found to be about + 180 degrees. Except for a transition region near $f_2 = 4$ kHz, the magnitude and direction of the phase shifts for any particular stimulus parameters were quite consistent from animal to animal. For example, for the reference parameter set ($f_2 = 8$ kHz, $f_2/f_1 = 1.28$, $L_1 \times L_2 = 55$ dB SPL) the magnitude was 160–190 degrees and the shift was in the same direction for all injections in all nine animals in this study. The fact that the phase angle between these two components does *not* vary randomly suggests that the generation mechanisms of the passive and active components are intimately related at the level of cochlear mechanics.

The presence of a relatively constant response between the sharp minima, especially when contrasted to the rapid variation of the active component, further suggests the presence of an underlying passive component which is relatively unaffected by EP change. Note, however, that there was often observed some change in this component with time during the 'flat minimum'. Further, this component is certainly not completely invulnerable (e.g., Rubel and Norton, 1991; Schmiedt and Adams, 1981; Whitehead et al., 1992a,b). After anoxia, for example, the CDT emission at moderate stimulus levels was typically observed here to first decrease to the passive level established by furosemide injection, but after several min to abruptly drop below the noise floor.

The existence of 'two components' in the CDT for midrange frequency ratios ($f_2/f_1 = 1.2$–1.3) has been previously suggested on the basis of several other lines of evidence, including the shapes of normal input-output relationships (Brown, 1987; Whitehead et al., 1990, 1992a), changes in emissions after ototoxic drug treatment (Brown et al., 1989; Whitehead et al., 1992b), changes post mortem and/or with sound exposure (Johnstone et al., 1990; Lonsbury-Martin et al., 1987; Rubel and Norton, 1991; Whitehead et al. 1992b), and changes with development (Norton and Rubel, 1990; Norton et al., 1991). However, from observations including smaller frequency ratios (Brown et al., 1987; Whitehead et al., 1992a), it was concluded that the active CDT component is not observable at frequency ratios near unity. By observing the vulnerability to furosemide, however, the active component is clearly present at low and moderate stimulus levels at *all* frequency ratios from 1.05 to 1.71. It is true that at ratios near unity, the active component in gerbils is able to dominate over a smaller intensity area, and is correspondingly weaker and more variable. The active component at near unity frequency ratios could easily be missed if only a few stimulus levels were monitored, and might not be detectable in particular individuals or in some species. Therefore, the results found here agree generally with previous proposals for the existence of two components (review: Whitehead et al., 1992a,b).

4.2. Interpretation: A simple summation model for the two components

A very simple model for the generation of emissions would be composed of an 'active' source which is affected by furosemide, added to a 'passive' source which is relatively unaffected. For the simplest model, suppose that only the amplitude of the active source varies with time after furosemide injection, and its phase angle is constant. If the difference in phase angle between the two sources is between 90 and 180 degrees (or between −90 and −180 degrees) there will be a minimum in the total emission when the ampli-

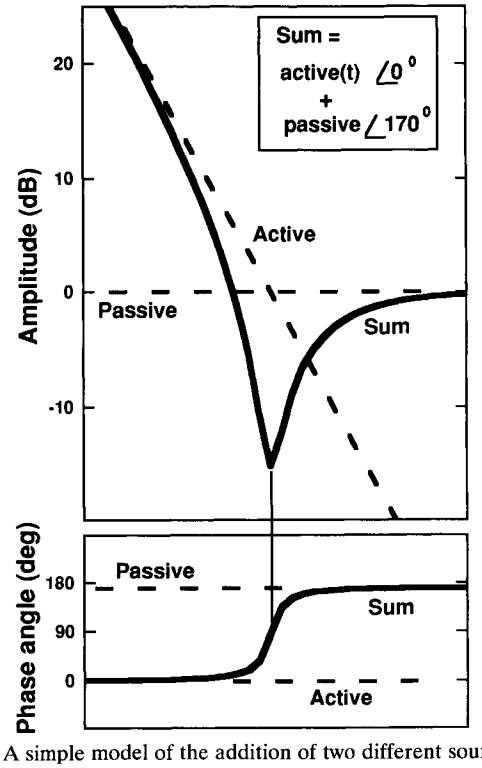

Fig. 11. A simple model of the addition of two different sources. The amplitude of the active source (dashed line) is assumed to decrease as shown after furosemide injection, while its phase angle is constant. This emission is added to a unchanging source with a different emission phase angle, in this case 170 degrees larger. The total emission is represented by the solid line. At the minimum in magnitude, the shift in the phase angle of the total is half the amount it will be when the active source is negligible.

tude of the active source is equal that of the passive source. At this point, the phase angle of the sum will be one-half the difference in phase angle between the two sources. As the amplitude of the active source decreases further, the amplitude of the sum rises again, with the total amplitude and angle quickly becoming equal to that of the passive source. The behavior of such a system is illustrated in Fig. 11, for a difference angle of 170 degrees.

It can be seen that the behavior is very much like that often observed, e.g., in Fig. 3. Note that in this simple model, there is a relationship between the total shift in angle and the depth of the sharp minimum. If the difference in phase angle between the two components is about 180 degrees, there will be a very sharp minimum. For shifts less than 90 degrees, there will be no sharp minimum at all, just the 'flat minimum'.

While this simple summation model successfully reproduces the general appearance of the phase shifts observed, there are some potential difficulties with it. First, it cannot account for phase shifts in excess of 180 degrees. However, such larger shifts have not been unequivocally observed. Second, with the magnitude of the angle between the two components usually observed, i.e., of the order of 180 degrees, it might be expected that the angle shift would occur equally often in either direction, and it has been found (e.g., Fig. 10b) that the phase angle shift occurred predominantly in the positive (leading) direction. In a similar vein, for parameters where the shift is found to be very close to 180 degrees, it might be expected that the direction of shift would at least vary from animal to animal, if not with different injections. However, this did not occur.

In spite of these difficulties, it is interesting that there is such good agreement in both magnitude and phase angle between the predictions of the simple summation model (Fig. 11) and our experimental observations (e.g., Fig. 3). The agreement suggests that both active and passive components must normally be present simultaneously. Further confirmation of the simultaneous presence of both components comes from comparing emissions at different distortion frequencies, e.g., the two odd components in the signal for $f_2/f_1 = 1.28$ in Fig. 8. When considering the magnitudes of the $2f_1 - f_2$ component and the $3f_1 - 2f_2$ component in the *same signal* following furosemide injection, there are occasions when the emission at one of these frequencies decreases to a passive component, while the other does not. Therefore, at the same time with the same stimulus the emission can be dominated by the passive component at one emission frequency but not at another.

This model also provides an explanation for the sharp 'crevasses' seen in some maps in Fig. 1. Near cancellation of the total signal due to a difference in phase angle between two nearly equal amplitude components has been previously suggested as an explanation for these structures, which appear as 'notches' in input-output functions (Brown, 1987; Norton et al., 1991; Whitehead et al., 1992a; Wiederhold et al., 1986).

4.3. Using DPOAE and furosemide injection as an assay of active processes

DPOAE have previously been extensively employed as a measure of cochlear function. Applications include the assessment of the effects of aminoglycoside toxicity (Brown et al., 1989; Whitehead et al., 1992b), noise damage (Franklin et al., 1991; Johnstone et al., 1990; Schmiedt, 1986; Wiederhold et al., 1986), development (Henley et al., 1989; Lenoir and Puel, 1987; Norton et al., 1991), genetic defects (Horner et al., 1985; Schrott et al., 1991), and normal hearing function (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Martin et al., 1990), as well as basic investigations of normal cochlear dynamics (Allen and Fahey, 1992, 1993; Brown and Gaskill, 1990; Brown and Williams, 1993). The data presented here suggest that using DPOAE in conjunction with furosemide injection in mammalian experimental animals can potentially provide an improvement in assaying the contribution of active processes to cochlear function.

Our parametric results suggest that it is best to avoid frequency ratios near unity for the CDT ($2f_1 - f_2$) and fifth order ($3f_1 - 2f_2$) terms. At the smaller ratios these emissions are quite variable; emission amplitudes tend to vary erratically with time, and between animals. The most consistent results are found at middle ratios around $f_2/f_1 = 1.2$–1.3, and at moderate stimulus levels, e.g., $L_1 \times L_2 = 55 \times 50$ dB SPL. Higher frequency ratios, or slightly higher stimulus levels, can also be employed, but it is found that the decrease to a flat minimum is not as consistently observed with these parameters. Noise contamination becomes a problem at maximum furosemide effect for much lower stimulus amplitudes.

It seems reasonable to form the working hypothesis, therefore, that DPOAE analysis using moderate stimulus frequency ratios can be usefully employed to monitor active processes in the cochlea, at least over the frequency range 1–16 kHz. With furosemide injection, the presence of the active process can be demonstrated by a shift in the emission patterns such as seen in Figs. 5 and 6. The abrupt phase shift, such as seen in Figs. 3 and 4, can be used to verify that the active component had been interrupted completely, and the passive component successfully uncovered. A failure to detect these characteristic changes in emissions with furosemide injection could tentatively be attributed to a absence of normally functioning active cochlear mechanisms at the stimulus frequencies.

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