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THE CHARACTERIZATION OF DISTORTION PRODUCT OTOACOUSTIC EMISSIONS IN THE SPRAGUE-DAWLEY RAT DURING DEVELOPMENT

by

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An independent study submitted in partial fulfillment of
the requirements for the degree of

Master of Science in Speech and Hearing

Emphasis in Audiology

Washington University
Department of Speech and Hearing

May 24, 2002

Approved by: Dwayne Simmons, Ph.D., Independent Study Advisor

ABSTRACT

The purpose of this project was to setup a system to measure distortion product otoacoustic emissions (DPOAEs) in the Sprague Dawley rat during development. The system and the stimuli were set up using hardware from Tucker Davis Technologies (TDT) and Etymotic Research and TDT software. The setup included sound level measurements in an Industrial Acoustics Corporation (IAC) sound booth with a Bruel and Kjaer (B&K) sound level meter. The inside walls and ceiling of the IAC booth were treated with acoustic foam tiles. To ensure the system and acoustical stimuli were functioning appropriately, a pilot study was conducted. Sprague Dawley rats were tested every five days from postnatal day fifteen to postnatal day forty. The stimulus tones for the pilot study were setup in two groups to test a variety of levels at a six combinations of frequencies. The stimulus tones were set up so that the levels of the first frequency (f1) and the levels of the second frequency (f2) were equal (0 dB difference) and then the levels of f2 were 10 dB SPL less than the level of f1 (10 dB difference). The pilot study suggests that the most robust distortion products in the Sprague Dawley rat are measured when the f2 frequency is 8 kHz. Contrary to expectations, the sets of tones with 0 dB difference produced more robust DPOAEs than the tones with 10 dB difference.

INTRODUCTION

Otoacoustic emissions (OAEs) were first reported by David Kemp (Kemp, 1978) in London (Norton and Stover, 1994). The OAEs reported by Kemp were found within the human auditory system and soon after he published his findings on OAEs in animals. OAEs are sounds generated within a normal functioning cochlea (Norton and Stover, 1994). They are faint sounds that can be measured in the ear canal with a low noise microphone (Allen and Lonsbury-Martin, 1993). OAEs can be either spontaneous or evoked. Spontaneous OAEs are narrow-band, low-level signals that occur naturally in about 50% of normal human ears (Norton and Stover, 1994). Evoked OAEs include transient evoked OAEs, stimulus frequency OAEs, and distortion product OAEs (DPOAEs). Transient evoked OAEs are elicited by brief clicks, stimulus frequency OAEs are elicited with long duration tones, and distortion product OAEs are elicited with two primary tones (Allen and Lonsbury-Martin, 1993).

DPOAEs are sounds that are measured when the ear is stimulated with two simultaneous pure tones (Hall, 2000). DPOAEs are a way of looking at cochlear function with a noninvasive approach (Liberman, 1996). The most prominent distortion product (DP) is the cubic difference tone that is measured at 2f1-f2 (Harris, Lonsbury-Martin, Stagner, Coats, and Martin, 1989). This DP is found at a level that is usually about 60 dB below the primary tone (Gelfand, 1998). DPOAEs are thought to analyze the functional integrity of the cochlea (Abdala, 2000) at the place along the basilar membrane that corresponds with the f2 frequency or the frequency that corresponds to the geometric mean of the two tones (Jimenez, Stagner, Martin, and Lonsbury-Martin, 1999). For measuring DPOAEs in the human ear, the most often used combination of levels for the f1 and f2 frequencies is 60 dB SPL and 50 dB SPL, respectively (Norton and Stover, 1994). Morphological development of the cochlea in humans is adult-like during the last trimester of pregnancy (Lavigne-Rebillard and Pujol, 1987, 1988) so it is virtually impossible to study development of the cochlea using a human model. Rodents are unique animals to use for hearing research since their cochlear development continues after birth until about postnatal day 16 through postnatal day 18 (Pujol, Lavigne-Rebillard, and Lenoir, 1998). DPOAEs are thought to arise from outer hair cells (OHCs) within the cochlea (Kiang NY, Moxon EC, and Levine RA, 1970). Therefore, using the rat model, it is possible to study the development of the cochlear amplifier by the use of DPOAEs. The cochlear amplifier involves mechanical feedback from

OHCs to the basilar membrane. The non-linearity of this mechanical system is such that with two-tone stimulation, DPOAEs are generated by this amplification process (Gaskill and Brown, 1990). The goal of this study was to set up a system to measure DPOAEs in rats and conduct a pilot study to measure the development of DPOAEs as a function of age.

METHODS

Booth Setup

A six by six and a half square foot Industrial Acoustics Corporation (IAC) single wall booth was erected in the basement of the Fay and Carl Simons Center for Biology of Hearing and Deafness at Central Institute for the Deaf in Saint Louis, Missouri. Sound level measurements were made using a Bruel and Kjaer (B&K) Sound Level Meter. Sound level measurements were made using 1/3-octave bandwidths at frequencies from 20 Hz to 20 kHz. Measurements were also made with broadband linear, C-weighted and A-weighted filters. It was determined that acoustical treatment should be added in order to reduce reverberation and low frequency noise inside the booth. The walls and ceiling were treated with 4-inch pyramidal melamine foam tiles (Figure 1). The tiles were adhered to the walls with Liquid Nails and connected to the ceiling using wood screws.

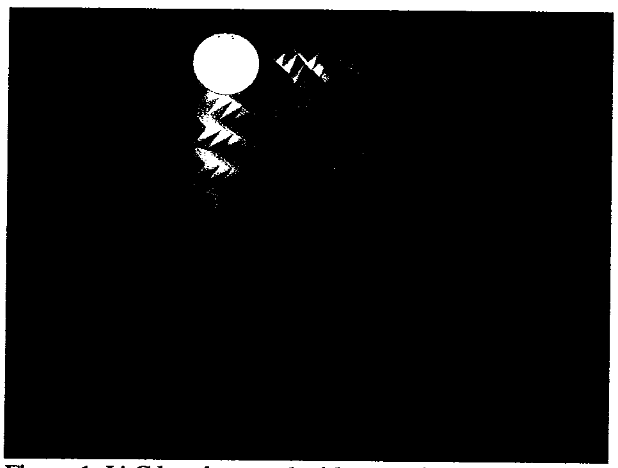


Figure 1 IAC booth treated with acoustic melamine foam tiles

Equipment

Tucker Davis Technologies (TDT) hardware and software were used to generate stimulus tones. The TDT software that includes SigCal32, SigGen32, and BioSig32 was run on a Windows based PC (Hewlett Packard Kayak XA). The signals generated by the TDT system were observed on a digital B+K oscilloscope. Due to the frequency range heard by rats (Fay, 1988), the stimulus tones were presented to the animal's ear using TDT high frequency transducers. An Etymotic Research ER10B+ low noise microphone and pre-amp were used to pick up the signal coming back in the ear canal. An adjustable animal stage was built to hold the transducers and the animal during testing and allow fine adjustments to be made in securing the probe in the animal's ear canal.

Calibration of System

Initially, the system was calibrated using the B&K Sound Level Meter (SLM). Pure tone signals generated with the TDT system were produced and presented to a pressure microphone on the B&K SLM through a .4 cc cavity. The levels produced by a 9-volt signal were measured and recorded at octave frequencies ranging from 1 kHz through 30 kHz. The recorded levels were then used to create a file to make necessary attenuation to the signals at each frequency. This was a tedious method and the TDT technical support personnel recommended using their SigCal32 software for system calibration. SigCal32 was used to present stimuli at a series of frequencies into a closed cavity. The amplitude of the signal coming back and being measured by the ER10B+ microphone was recorded and plotted for each frequency on a graph in the SigCal32 software. Once the plot had been made in the software, a schedule file could be created to make necessary attenuation at each frequency using the programmable attenuators that were part of the TDT hardware. A calibration was run for the f1 frequencies and the f2 frequencies separately. The Schedule File created for each set of frequencies was then attached to the stimulus files in the TDT SigGen32 software.

Pilot Study Animal Preparation

Sprague Dawley rats from postnatal day 15 (p15) to p40 were used to measure DPOAEs as a function of age. The animals were initially anesthetized intraperitoneal (i.p.) using a ketamine/xylazine cocktail (4 mg/ml ketamine, 21 mg/ml xylazine, 0.1 ml/20 g total body weight). A quarter of the original dose was re-administered to maintain anesthesia as needed. An antibiotic ointment was used on the animal's eyes to prevent them from drying out during testing. The animals were kept on a heating pad during testing so that its body temperature could be maintained at 37°C ±1° (Figure 2). A YSI 4600 Precision Thermometer was used to ensure that the animal's core temperature was maintained during testing (Figure 3). The Central Institute for the Deaf Institutional Animal Care and Use Committee and the Washington University Biosafety Committee approved all experimental protocols.

Figure 2 Deltaphase heating pad from Braintree Scientific

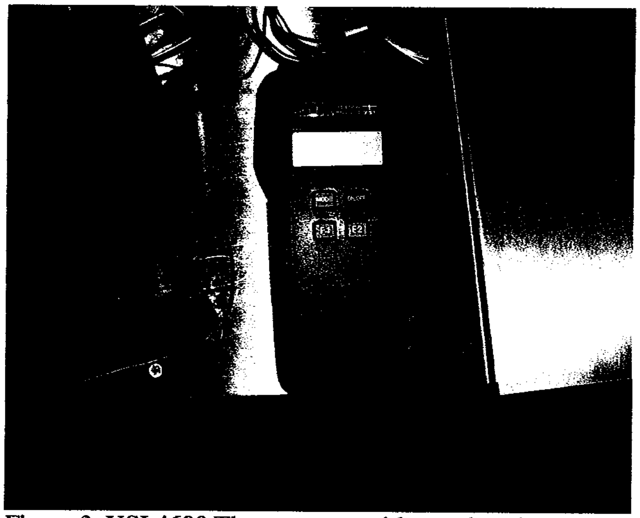


Figure 3 YSI 4600 Thermometer with rectal probe

Stimulus Setup

The stimulus tones were created separately using the TDT SigGen32 software. Each tone contained a set of variables. The first set of tones had variables that controlled the frequencies and the levels of the tones. The second set of tones had variables that controlled the frequencies, the levels, and the amount of attenuation to be applied to each programmable attenuator on the TDT hardware. The length of the stimulus tones for both groups was 51 ms with a 10 ms cosine ramp at the onset and a 10 ms ramp at the offset of the stimulus (Figure 4). The stimulus was presented continuously with a sampling rate of 9 us.

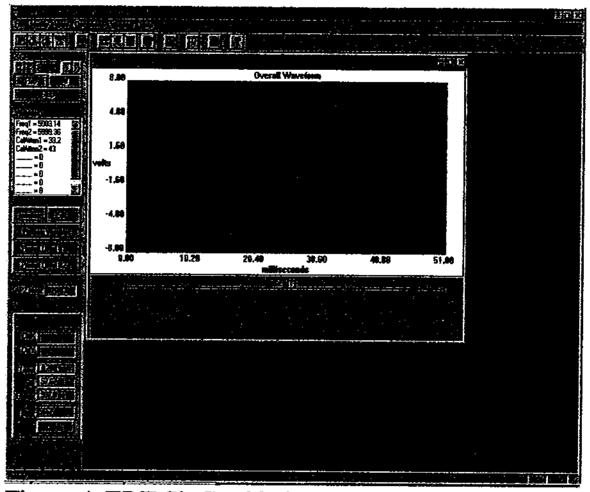


Figure 4 TDT SigGen32 signal

Automatic cursors were setup in the BioSig32 software to mark the f1 frequency, the f2 frequency, and the cubic distortion product of 2f1-f2. The cursors allowed the frequency and level to be recorded so that the data could be analyzed. A cursor was also placed at 100 Hz above and below the distortion product to estimate the noise floor of the system compared to the level of the distortion product.

RESULTS

Booth Acoustics

The overall sound level measured in the IAC booth before the treatment of acoustic foam was about 85 dB SPL with most of the noise being measure below 1kHz. After the acoustic foam treatment, the levels measured at each frequency did not show significant change. However, the overall sound level was decreased by 12 dB. A graph of the changes at octave frequencies can be seen in Figure 5.

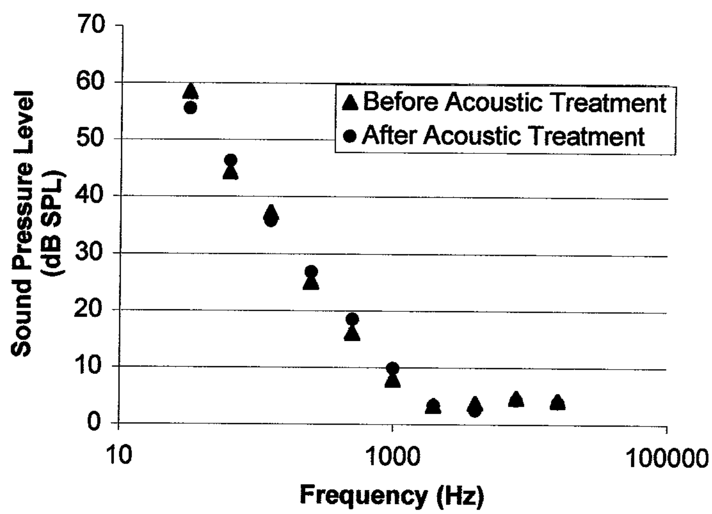


Figure 5 The sound pressure level measured at each octave frequency in 1/3-octave bandwidths from 20 to 16,000 Hz. This figure shows only the measurements at the octaves as a representative sample.

Calibration

The TDT system was calibrated using the TDT SigCal32 software. A calibration was made on each animal on each day using the animal's ear as the calibration cavity. To choose frequencies to test that did not fall in the area of standing waves, a calibration was run into two small cavities (0.2 ml and 0.5 ml) (Pearce, Richter, and Cheatham, 2001). The frequencies in which standing waves occurred were avoided when choosing sets of frequencies to be used for measuring DPOAEs (Figure 6).

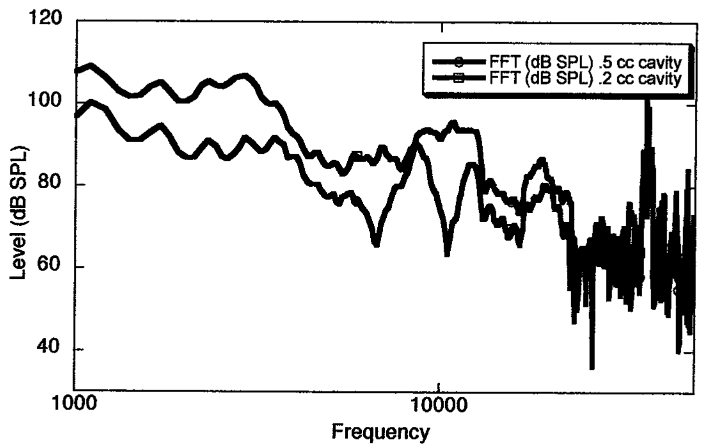


Figure 6 shows the measurements made in a closed cavity of .5 cc and .2 cc. The area in which standing waves occurred was avoided when pairs of frequencies were selected to measure DPOAEs.

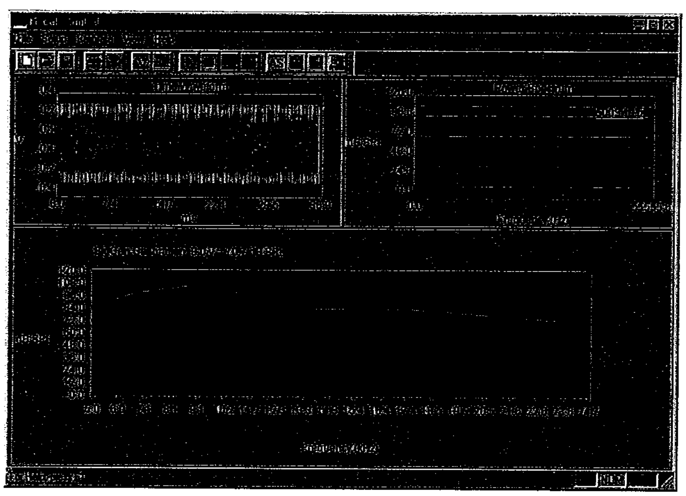
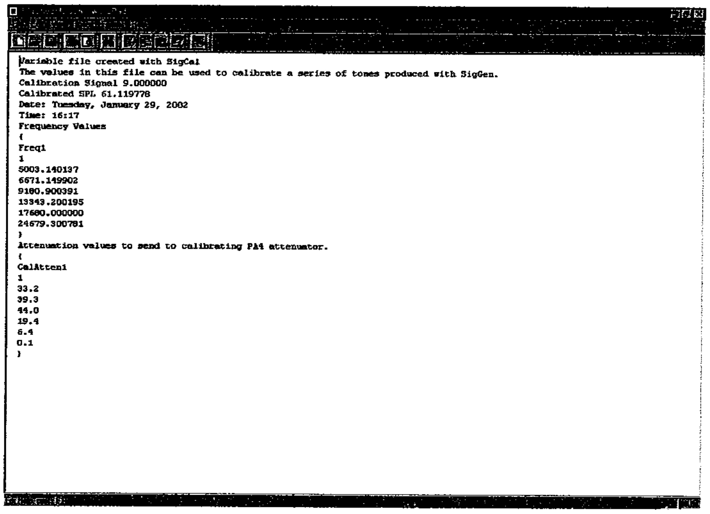


Figure 7 TDT SigCal32 calibration curve measured in an animal's ear cavity. This calibration was then saved as a variable schedule file (Figure 8) and attached to each stimulus file in SigGen32

Once the six pairs of frequencies were selected, the TDT SigCal32 calibration software was set up to test the calibration for each frequency to be presented. The SigCal32 program would create a schedule file to be attached to each stimuli file in SigGen32. (Figures 7 & 8)



```
Variable file created with SigCal
The values in this file can be used to calibrate a series of tones produced with SigGen.
Calibration Signal 9.000000
Calibrated SPL 61.119778
Date: Tuesday, January 29, 2002
Time: 16:17
Frequency Values
{
Freq1
1
5003.140137
6671.149902
9180.900391
13343.200195
17680.000000
24679.300781
}
Attenuation values to send to calibrating PA4 attenuator.
{
CalAtten1
1
33.2
39.3
44.0
19.4
6.4
0.1
}
```

Figure 8 Example of a schedule file created using SigCal32. The first list of values (Freq1) is the f1 frequencies (f2 frequencies are in a seperate file). The second list of values (CalAtten1) is the amount of attenuation to be applied at each frequency.

Pilot Study Stimulus Setup

The first set of tones was setup to maintain constant levels while varying frequency. The levels of the first frequency (f1) and the second frequency (f2) were maintained at 60 dB SPL and 50 dB SPL, respectively. The ratio of f1:f2 was 1.22:1 (Gaskill and Brown, 1990) and the f2 frequencies were equal to 6, 8, 11, 16, 21.2, and 29.6 kHz.

Frequency f1 (Hz)	Frequency f2 (Hz)	Level f1 (dB SPL)	Level f2 (dB SPL)
5003.14	5999.36	60	50
6671.15	7999.51	60	50
9180.9	11009	60	50
13343.2	16000.1	60	50
17680	21200.5	60	50
24679.3	29593.5	60	50

Table 1 Shows the frequencies and levels used in the first set of files which swept through a range of frequencies while keeping the levels constant with a 10 dB difference.

The second set of tones was setup to vary frequency and amplitude. The same frequency sets were used as in the first set of tones. For each frequency set, the levels of f1 and f2 [L(f1) and L(f2), respectively] were varied in an alternating manner so that L(f1) and L(f2) were first set equal to each other and on the subsequent trial, L(f2) was set to 10 dB SPL less than f1. Next, the level of f1 was decreased by 5 dB SPL and L(f2) was again set equal to L(f1). On the subsequent trial, L(f2) was again decreased by 10 dB SPL. This pattern was used with levels of f1 ranging from 60 to 40 dB SPL (Table 2)

Frequency f1 (Hz)	Frequency f2 (Hz)	Level f1 (dB SPL)	Level f2 (dB SPL)
5003.14	5999.36	60	60
5003.14	5999.36	60	50
5003.14	5999.36	55	55
5003.14	5999.36	55	45
5003.14	5999.36	50	50
5003.14	5999.36	50	40
5003.14	5999.36	45	45
5003.14	5999.36	45	35
5003.14	5999.36	40	40
5003.14	5999.36	40	30

Table 2 gives an example of the way the levels of the tones were swept at each set of frequencies. The sequence of level sweeps was repeated at all six sets of frequencies.

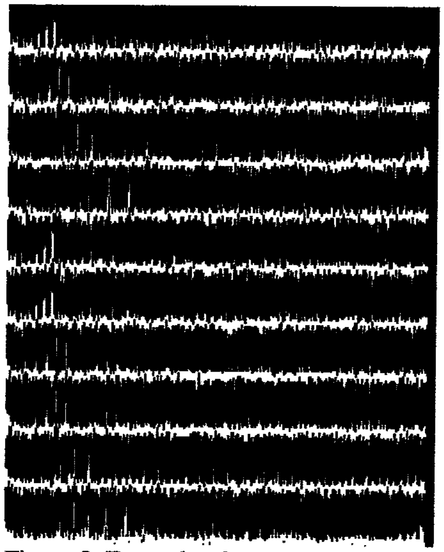


Figure 9 Example of an FFT. Frequency is on the abscissa and amplitude (level) is on the ordinate. The first four FFTs are showing the sweep in frequency. The last six FFTs are samples of the sweep in level with 0 dB difference and 10 dB difference (alternating) as frequency is increased.

As the BioSig32 software ran through each set of stimuli and picked up the sound coming back, the sound was analyzed and plotted in a Fast Fourier Transform (FFT). The FFT showed the noise floor of the system, as well as the f1 frequency, the f2 frequency and the distortion product at 2f1-f2 (Figure 9).

Pilot Study Frequency Results

The DPOAEs of Sprague Dawley rats from p15 to p40 were measured. The purpose of this study was to see if the amplitude of the distortion product would increase as the rat reached cochlear maturity at approximately p17-p20. On the day in which the rats were 15 days old, the attempt to measure DPOAEs was somewhat unsuccessful due to the inability to fit the ER10B+ probe microphone into the animal's ear canal. One animal was fit successfully with the probe, but the animal died before testing could be completed. Therefore, limited data was collected on p15. On p20, the animal died during testing. This was unfortunate, however the animal was retested while it was deceased and was used as a control. This data shows that what was being measured was actually DPOAEs. The data from the animal while it was alive and after can be seen in Figure 10.

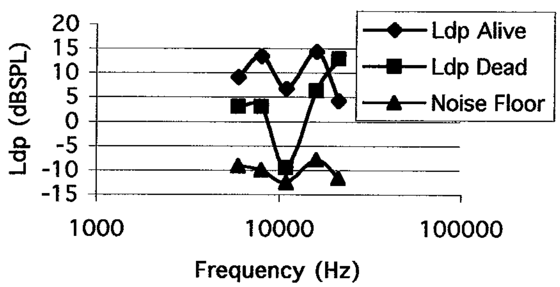


Figure 10 shows the DPOAEs measured in the same p20 rat while it was alive and after it died. The average noise floor measured during the testing was also plotted. The most robust change in the level of the DPOAE was at 11 kHz. The DPOAE level at 21.2 kHz increased after the animal was deceased, therefore the data from 21.2 kHz was disregarded for the remainder of the study.

The data in Figure 10 shows that the DPOAEs measured in the rat on p20 while it was alive was most robust at 8 kHz and 16 kHz. The most robust change in the amplitude of the DPOAEs after the rat died was at 11 kHz. The DPOAEs measures at 8 kHz were consistently more robust than at any other frequency. The lower line on the graph represents the average noise floor for the measurements while the animal was dead and alive. The DPOAEs measured when the F2 frequency was equal to approximately 21.2 kHz showed an increase in amplitude after the

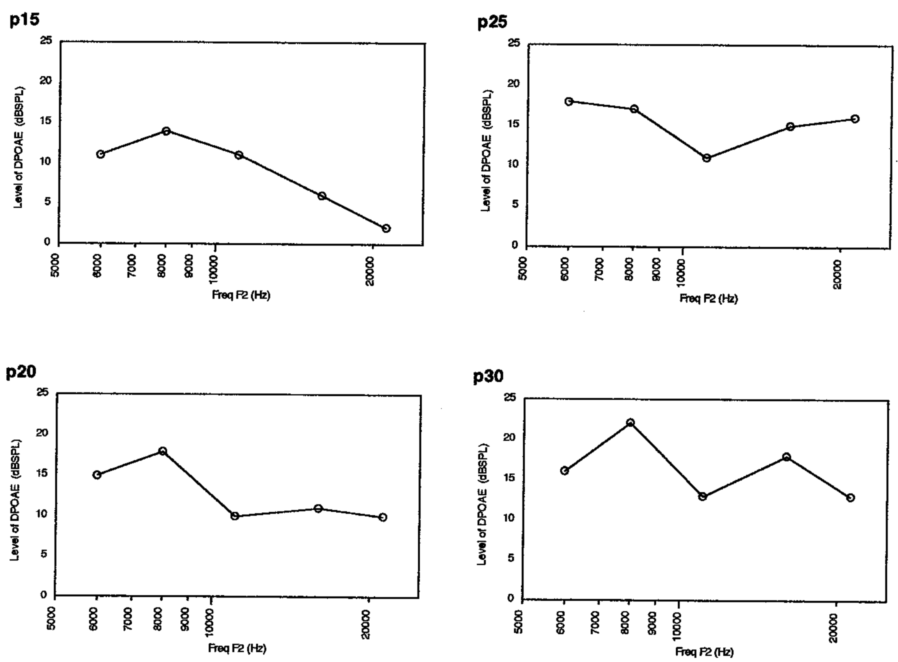


Figure 11 shows that the most consistent f2 frequency for robust DPOAEs is 8 kHz. The only age that is not consistent with this is p25. However, at p25, 8 kHz is the second highest frequency.

animal died. A comparison was made of the level of the DPOAE as a function of f2 frequency at each age (Figure 11).

The levels of the DPOAEs were measured as a function of age and compared by f2 frequency. The best DPOAE at each frequency was plotted for each day of testing. The results of this comparison can be seen in Figure 12.

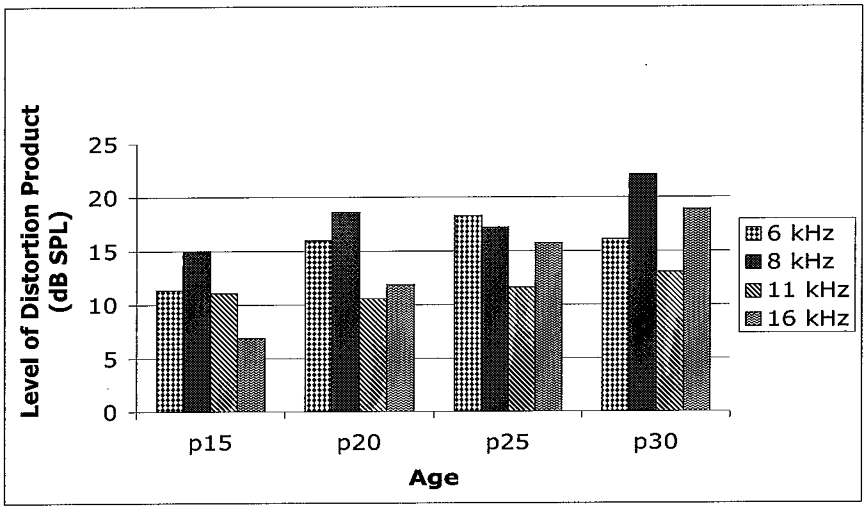
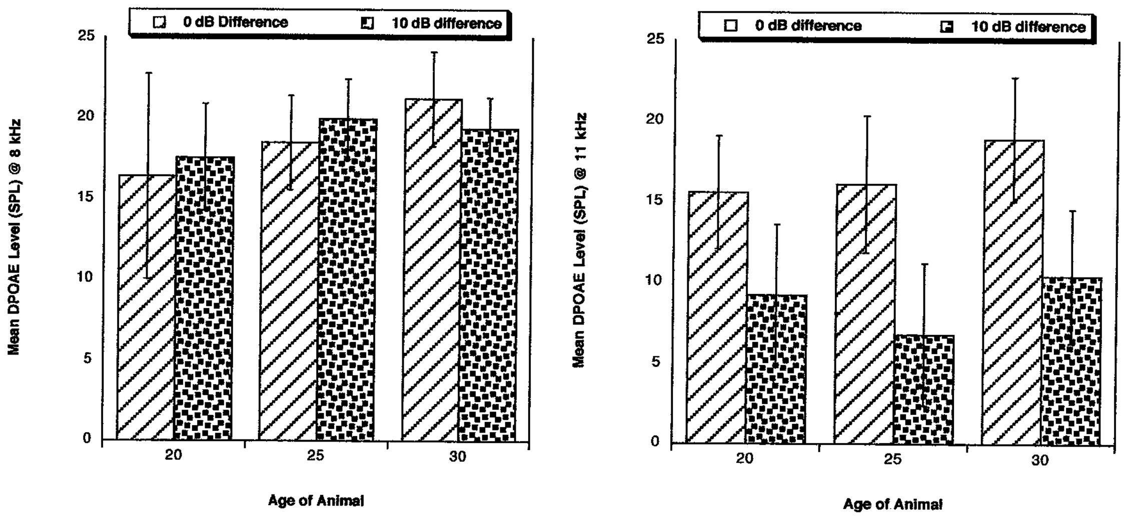


Figure 12 shows the change in the level of the DPOAEs as a function of age and frequency. The most robust DPOAE was measured at 8 kHz on p30. Steady growth of DPOAEs occurred at 16 kHz. The DPOAEs measured at 11 kHz also had steady growth, however the amplitude of the DPOAEs was not as robust.

Pilot Study Level Results

A comparison was made of the mean DPOAE level as a function of age and level difference of the f1 and f2 frequencies. The comparison showed that at 8 kHz, the levels of the DPOAEs measured when the stimuli were presented at the same levels were not significantly different. The results at 11 kHz were significantly different. The DPOAEs measured when the stimuli levels were f1=f2=60 (0 dB difference were significantly more robust than the 10 dB difference stimuli (Figure 13). The data in Figure 13 shows that at 8 kHz there was no significant difference in the levels of the 0 dB difference and the 10 dB difference. The data at 11 kHz however, was significantly different with the 0 dB difference data producing more robust DPOAEs at all ages.



The graphs in Figure 13 show the mean level of the DPOAEs as a function of age. The striped bars show the levels when a two stimulus tones were presented at the same level (0 dB difference) and the dotted bars show the levels when the stimulus tones were presented with the level of f1 10 dB above the level of f2. The data included in these graphs are the average data from the f1/f2=60/60 at f2=8 kHz and 11 kHz and the f1/f2=60/50 at f2 = 8 kHz and 11 kHz. Data from one rat at p30 was eliminated from the averages because its DPOAEs were equal to 0 and greatly skewed the data.

DISCUSSION

Frequency Results

The setup of this system to measure DPOAEs in rodents was successful. We were able to measure DPOAEs in rodents as a function of age. The most robust distortion products were measured when the frequency of the f2 tone was equal to 8kHz. This was interesting to know since the frequency range of hearing in the Sprague Dawley rats quite different than that of humans. Rats hear frequencies ranging from 1 kHz to about 80 kHz (Fay, 1988). The system measuring the DPOAEs was able to test frequencies up to about an f2 of 18 kHz successfully. The set of frequencies that had an f2 frequency of 21.2 and 29.6 kHz had a large amount of distortion in the cavity and was eliminated from the results used to draw conclusions.

Level Results

The levels of the tones that caused the most robust distortion product at f2=11 kHz was the 0 dB difference sets of stimuli. This was not the expected result. In humans, the most robust DPOAE occurs with a 10 dB difference.

Calibration Issues

There was some difficulty getting the tones to be presented at the levels that were desired. While setting up the calibration procedure, the technical support was required on multiple occasions. The stimuli being produced for f1 and f2 were delivered into the ear canal by a probe with also contained a microphone to pick up the o stimuli tones and the distortion product coming back in the ear canal. The levels of the f1 and f2 frequencies were sometimes accurate, but were often off by as much as five to seven dB SPL. This problem seemed to have no obvious solution and therefore we proceeded with the system the way it was. The levels being picked up by the microphone in the ear canal may have been off because of the fact that they were not the levels being presented, but the levels coming back. This may account for the difference in level.

FUTURE RESEARCH

The data found in this pilot study is very preliminary. Future study should include similar research on larger number of animals over a longer period of time so that more stable conclusions can be drawn. It has also been speculated that as frequency increases, the ratio of f1:f2 may need to be varied as the frequency increases (Mills and Rubel, 1997). The inconsistency of results measured at the 0 dB difference and 10 dB difference suggests that additional research should be conducted to determine the level combination which produces the most robust DPOAEs in rodents.

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