DISTORTION-PRODUCT OTOACOUSTIC EMISSIONS IN THE COMMON MARMOSET (*CALLITHRIX JACCHUS*): PARAMETER OPTIMIZATION

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Abstract

Distortion-product otoacoustic emissions (DPOAEs) were measured in a New World primate, the common marmoset (*Callithrix jacchus*). We determined the optimal primary-tone frequency ratio (f_2/f_1) to generate DPOAEs of maximal amplitude between $3 – 24$ kHz. The optimal f_2/f_1, determined by varying f_2/f_1 from $1.02 – 1.40$ using equilevel primary tones, decreased with increasing f_2 frequency between $3 – 17$ kHz, and increased at 24 kHz. The optimal f_2/f_1 ratio increased with increasing primary-tone levels from $50 – 74$ dB SPL. When all stimulus parameters were considered, the mean optimal f_2/f_1 was $1.224 – 1.226$. Additionally, we determined the effect of reducing L_2 below L_1. Decreasing L_2 below L_1 by 0, 5, and 10 dB ($f_2/f_1 = 1.21$) minimally affected DPOAE strength. DPOAE levels were stronger in females than males and stronger in the right ear than the left, just as in humans. This study is the first to measure OAEs in the marmoset, and the results indicate that the effect of varying the frequency ratio and primary-tone level difference on marmoset DPOAEs is similar to the reported effects in humans and Old World primates.

Keywords

OAE; DPOAE; parameter optimization; primary-tone frequency ratio; primary-tone level difference; animal model; primate; marmoset

Introduction

Stimulating the ear with two pure tones, or primary tones, at frequencies f_1 and f_2 (with $f_1 < f_2$) can result in the generation of a number of nonlinear distortion-product otoacoustic emissions (DPOAEs) having frequencies algebraically related to the primary-tone frequencies. The strongest and most commonly examined of these nonlinear products is the cubic DPOAE, which has a frequency equal to $2f_1-f_2$. In humans, cubic DPOAEs are presumed to arise from the micromechanical activity of outer hair cells (Kemp, 1978) at two distinct places. They are generated near the f_2 region on the basilar membrane (e.g., Kim *et al.*, 1980; Brown & Kemp, 1984; Martin *et al.*, 1987; Harris *et al.*, 1992) and are re-emitted at the location of the DPOAE frequency (f_{DP}) region (e.g., Brown *et al.*, 1996; Gaskill & Brown, 1996; Talmadge *et al.*, 1998; Mauermann & Kollmeier, 1999; Shera & Guinan, 1999). DPOAEs then are propagated

backwards along the basilar membrane, through the middle ear, to the external auditory canal where they can be recorded with a sensitive probe microphone. The relative amplitude of these emissions at specific frequencies may reflect the health of the basilar membrane at specific loci, and if so, DPOAEs provide a non-invasive method of measuring cochlear health.

DPOAEs have been studied in numerous non-primate species including (but not limited to) mice (e.g., Horner *et al.*, 1985; Schrott *et al.*, 1991; Parham, 1997; Varghese *et al.*, 2005), rats (e.g., Brown, 1987; Khvoles *et al.*, 1998), gerbils (e.g., Schmiedt, 1986; Brown & Kemp, 1984; Brown, 1987; Norton & Rubel, 1990; Mills & Rubel, 1994) guinea pigs (e.g., Canlon *et al.*, 1983; Brown, 1987; Brown & Gaskill, 1990a), chinchillas (e.g., Kim, 1980; Zurek *et al.*, 1982), rabbits (e.g., Lonsbury-Martin *et al.*, 1987; Martin *et al.*, 1987; Whitehead *et al.*, 1992a,b), dogs (e.g., Sockalingam *et al.*, 1998), spotted hyenas (McFadden *et al.*, 2006b), cats (e.g., Kim, 1980; Wiederhold *et al.*, 1986; Schmiedt, 1986), and bats (e.g., Kössl, 1994) to determine the effects of noise, drugs, anesthesia, temperature, age, and prenatal androgen exposure on the cochlea. However, these species exhibited DPOAEs that were approximately 30 – 40 dB SPL greater than those measured in humans (for a review, see Probst *et al.*, 1991). Due to the inter-species differences, some investigators (Martin *et al.*, 1988; Lasky *et al.*, 1995; Park *et al.*, 1995) have suggested that non-human primates may serve as a more appropriate model for cochlear function, though Brown and colleagues (Brown, 1987; Brown & Gaskill, 1990a; Gaskill & Brown, 1990) have shown these species differences to be most pronounced with high-level primary tones and less evident with low- to moderate-level primary tones. By contrast, the effect of f_2/f_1 on DPOAE level (L_{DP}) varies considerably across species (Brown, 1987; Brown & Gaskill, 1990a), and the optimal f_2/f_1 is larger in rodents and rabbits (1.25 – 1.30) (Brown, 1987; Whitehead *et al.*, 1992) than in humans (1.22 – 1.23) (e.g., Harris *et al.*, 1989; Gaskill & Brown, 1990; Nielsen *et al*., 1993).

Indeed, DPOAEs in the rhesus monkey are more similar to human DPOAEs than are other laboratory animals (Martin *et al.*, 1988; Park *et al.*, 1995; Lasky *et al.*, 1995, McFadden *et al.*, 2006a). The strength of DPOAEs in rhesus monkeys can be approximately 45 – 60 dB SPL below (equilevel) primary-tone levels (McFadden *et al.*, 2006a), while human DPOAEs are approximately 60 dB SPL below primary-tone levels (for a review, see Probst *et al.*, 1991). Lasky *et al*. (1995) observed DPOAEs in rhesus monkeys that were 10 – 15 dB SPL stronger than those measured in humans. The mean optimal f_2/f_1 ratio in rhesus monkeys is 1.21 (Lasky *et al.*, 1995) compared to 1.22–1.23 in humans (Harris *et al.*, 1989; Gaskill & Brown, 1990; Nielsen *et al*., 1993). Decreasing L_2 below L_1 by 5 and 10 dB SPL had little effect on DPOAE levels in rhesus monkeys (Park *et al.*, 1995) and humans with healthy ears (Whitehead *et al*., 1995).

For comparative purposes, we aim to determine whether cochlear function is similar in a New World primate, the common marmoset. This species has emerged as a model for biomedical research in reproductive biology (e.g., Tardif *et al.*, 2005; Power *et al.*, 2006), behavioral research (e.g., Sieden, 1957; Barros *et al.*, 2007), and auditory function (e.g., Bendor & Wang, 2007; Bartlett & Wang, 2007; Schnupp *et al.*, 2006; de la Mothe *et al.*, 2006a, b). However, most auditory research in marmosets has focused on the central nervous system, particularly the auditory cortex, and only a few researchers have investigated the auditory periphery in this species (Wever & Vernon, 1961; Spatz & Löhle, 1995; Spatz, 1999). Sieden (1957), using a shock-avoidance technique, obtained behavioral audiograms from five marmosets (4 females and 1 male). These marmosets responded to pure tones as low as 100 Hz and as high as 25 – 37 kHz. Wever & Vernon (1961) measured cochlear potentials at frequencies up to 100 kHz in four (3 females, 1 male) of these same five marmosets. Spatz & Löhle (1995) showed that the marmoset cochlea takes about 2.5 turns, the same as in humans. To our knowledge, no one has systematically investigated cochlear function in this species. As a first step in this direction we have made detailed measurements of DPOAEs in the marmoset by varying stimulus

parameters to determine the extent to which the response is similar to those reported for humans and Old World monkeys. Our results show that DPOAEs in the marmoset respond to these variations in a manner similar to humans and Old World monkeys. Therefore, this paper serves as a starting point for the study of OAEs in this species.

Materials and Methods

Subjects

DPOAEs were measured in 6 male and 6 female common marmosets ranging between 1.3 and 3.2 years of age (mean = 2.05 years). Subjects were located at the Southwest National Primate Research Center (SNPRC) at the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, TX, housed either in group enclosures as family units or individually in rooms containing multiple enclosures. For four female marmosets, the optimal f_2/f_1 and $L_1 - L_2$ conditions were performed on separate days. For all male and two female marmosets both conditions were performed in a single session, permitting the same microphone placement for all measurements. All testing was conducted in a small procedure room at the SNPRC. Subjects were anesthetized with intramuscular injections of ketamine hydrochloride (Ketaset®, 10mg/ kg) and butorphanol tartrate (0.5 mg, regardless of weight). A maintenance dose of ketamine was required after an hour to minimize movement in some male subjects. The subject was placed on its side during recordings. The subject's temperature was maintained during recordings with an adjacent non-electrical heat source and monitored with a digital rectal probe thermometer at the beginning and end of each session. The average temperature change was minimal ($-1.68 \pm 1.49 \, °F$). These procedures were approved by the SFBR Institutional Animal Care and Use Committee (IACUC; CJ 10441).

Procedures and equipment

A probe microphone/loudspeaker system with two loudspeaker ports (ER10B+, Etymotic Research, Inc.) was fitted tightly into the ear canal with a rubber immittance tip. Two loudspeakers (EC-1, Tucker-Davis Technologies, Inc.), with flat frequency response from 4 – 100 kHz, were connected to the ER10B+ ports. Acoustic stimuli were delivered from an analog input/output (I/O) device (PXI-4461, 24-bit ADC, National Instruments, Inc.), through an electrostatic speaker driver (ED-1, Tucker-Davis Technologies, Inc.), to the EC-1 speakers. Each primary tone was delivered through a separate loudspeaker to avoid the generation of intermodulation products. Control measurements were performed with the microphone inserted into a 1-ml syringe (volume = 0.10 ml). No spurious intermodulation products generated by the electronics or by the acoustics of the canal were detectable at the DPOAE frequency in these control recordings.

The OAE response output from the ER10B+ microphone was amplified by 20 dB by a low-noise preamplifier (Etymotic Research, Inc.) and digitized at a 50-kHz sampling rate by the PXI-4461 module. The data-acquisition module was housed in a mobile chassis (PXI-1036DC, National Instruments, Inc.) and connected to a laptop computer (Latitude D800, Dell, Inc.) through the PC CardBus slot (PXI-8310 StarFabric® interface, National Instruments, Inc.). Custom software for acquiring DPOAEs was written in LabVIEW (National Instruments, Inc.) by one of the authors (E.G.P).

A modified CUBDIS procedure (Allen, 1990a) was used to estimate the level of the DPOAE. This procedure has been modified from that previously described by McFadden *et al.* (2006a, b). Briefly, the initial calibration procedure, which measures the frequency response of the ear canal to a single click, gave us information about the position of the probe tip in the ear canal. A smooth frequency response indicated a tight seal in the ear canal, and any deviation prompted us to reposition the probe. After ensuring a tight fit of the probe, two primary tones

at specified frequencies and levels were presented continuously to one ear, and the response was simultaneously recorded for 4-sec from the stimulated ear. Primary tones were calibrated at each frequency to produce sound output at 65 dB SPL in the subject's ear canal. The frequencies of the primary tones were chosen such that each segment of 1028 sample points contained an integral number of cycles (as did the DP component). The f_2/f_1 was as close to the desired f_2/f_1 as this sampling constraint allowed. Averaging in the time domain was performed using 2048-point segments of the waveform with 50% overlap. This procedure afforded a reduction in the noise floor level, and the 2048-point FFT analysis improved the frequency resolution, permitting testing at values of f_2/f_1 nearer to 1.0. The initial 2048 points and the strongest 15% of the other segments were discarded (to remove possible artifacts) and the remaining segments (about 165) were averaged to produce a waveform of 2048 points. This waveform was used to compute a power spectrum. The power in the frequency bin containing the DP was then converted to SPL and taken as the L_{DP}. The strengths of the primary tones were similarly determined. The noise floor was estimated by averaging six adjacent frequency bins above and below the f_{DP} (separated from f_{DP} by at least 3 frequency bins). Also recorded in each case were the measured levels of f_1 and f_2; this permitted detection of conditions where the primary tones' actual levels deviated from their target values.

Frequency ratio (f_2/f_1) optimization

DPOAEs were measured in the right ears of 12 (6 male, 6 female) marmosets. The left ear was also tested in a subset of these subjects (3 male, 2 female), so the total number of ears tested was 17. Right ears were tested first in every case. Seven f_2 frequencies were selected in 0.5-octave spacing covering the range 3 – 24 kHz. For each value of f_2, f_1 was varied such that the f_2/f_1 ranged from 1.02 – 1.40 in 0.04 increments, except between 1.18 – 1.24 where step size was 0.02. At each f_2/f_1 ratio equilevel primary tones were presented in 6-dB increments between 50 – 74 dB SPL. DPOAE levels were averaged across all ears at each stimulus parameter and the f_2/f_1 which elicited the strongest mean L_{DP} was taken as the optimal value.

The effect of decreasing L_2 below L_1

The effect of primary-tone level difference ($L_1 - L_2$) on the L_{DP} was determined in the same 12 subjects described above (n = 18 ears). Right ears (6 male, 6 female) were tested before left ears (2 male, 4 female) in each case. The same f_2 frequencies as above were used here. We chose an $L_1 - L_2$ paradigm in which L_1 remained constant and L_2 was decreased such that $L_1 - L_2 = 0$, 5, and 10 dB SPL. The f_2/f_1 was held constant (approximately 1.21) throughout. At each f_2, a value of $L_1 - L_2$ was selected, and an input/output (I/O) function was obtained with L_1 presented in 6-dB increments between 50 – 74 dB SPL. The values of L_{DP} were averaged across all ears at each $L_1 - L_2$, and these mean DPOAE values were used to produce I/O functions (L_{DP} vs. L_1) and DPgrams (L_{DP} vs. f_2). Examination of theses I/O functions revealed them to be linear over the range of L_1 values from 50 – 68 dB SPL. Linear-regression analysis was performed using this range to determine the slopes of the I/O functions.

Normative DPOAE audiograms and Input/Output functions

When evaluating normative marmoset DPOAE levels for any ear or sex differences, we will report the effect size (*d*) in place of statistical significance because the sample sizes were small. We calculated effect size by dividing the difference between the two means being compared by the square root of the weighted mean of the two variances. Cohen (1992) has suggested that effect sizes of 0.2, 0.5, and 0.8 should be considered small, medium, and large, respectively.

Data Omission

In some subjects there were calibration errors at higher f_2/f_1 ratios which resulted in frequency ranges (12 and 24 kHz) in which not all f_2/f_1 ratios were presented (see Discussion section).

For these subjects, we did not include the incomplete frequency ranges in our final analysis to determine the optimal f_2/f_1 ratio. In one subject DPOAEs were not significantly greater than the noise floor at 24 kHz, so the data for this frequency were not included in our analysis. Data for another subject were excluded from the 24-kHz analysis as well because the voltage input (to the loudspeaker) required to produce 24-kHz tones was significantly larger than at all other frequency ranges in the same subject (and all other animals at 24 kHz), suggesting that, for this subject, the probe tip may have shifted between the 17- and 24-kHz measurements.

When f_2 was at or below 8.5 kHz, an f_2/f_1 ratio of 1.02 (1.06 at 3 kHz) placed the f_1 primary-tone frequency within nine frequency bins of the f_{DP}, thereby including it in the average noise floor estimate. Because this resulted in a gross overestimation of the noise floor, we have omitted the affected data points from the appropriate figures.

Results

Frequency ratio (f_2/f_1) optimization

In general, the mean f_2/f_1 functions were bandpass in shape: as f_2/f_1 increased, the average DPOAE level increased to some maximum (usually occurring at mid-f_2/f_1 ratios) and then decreased as the f_2/f_1 was further increased. This was evident in the mean f_2/f_1 functions shown for 6 – 17 kHz (Figs. 1, 2) and in the individual f_2/f_1 functions (described later), although the f_2/f_1 which elicited the most robust DPOAE varied according to ear, sex, primary-tone level, and frequency. Table 1 summarizes the mean optimal f_2/f_1 at each f_2 frequency and primary-tone level.

At 4 kHz (Fig. 1) the bandpass shape is seen most clearly at the lowest level of the primary tones, and as the primary-tone levels were increased from 50 – 74 dB SPL we observed less of an effect of f_2/f_1 on the average L_{DP}. For example, with 68-dB SPL primary tones, the mean L_{DP} elicited with f_2/f_1 ratios between 1.176 and 1.242 all were within 1.0 dB (with the maximum L_{DP} occurring at 1.176).

At the lowest and highest f_2 values that we tested (3 and 24 kHz), the f_2/f_1 functions did not exhibit the characteristic bandpass shape that was seen for all other frequency ranges. At 3 kHz (Fig. 1), the mean L_{DP} increased with increasing f_2/f_1, with an initial peak at $f_2/f_1 = 1.196$ (1.173 for 74 dB SPL primary tones). In the mean functions, a small dip in L_{DP} was observed following this peak, after which the DPOAE level increased again as the f_2/f_1 ratio was further increased. At 24 kHz, the effect of f_2/f_1 on the L_{DP} differed greatly between individual subjects. None of the individual f_2/f_1 functions (at 24 kHz) was similar in shape to the mean f_2/f_1 function (data not shown).

Each plot of the mean DPOAE levels elicited by 62-dB SPL primary tones shown in Fig. 1 was selected and replotted in Fig. 2 to indicate the typical amount of inter-subject variability we observed at each point. Although the inter-subject variability was relatively high in most frequency ranges, the individual f_2/f_1 functions typically followed a similar pattern within a frequency range and differed mainly in overall DPOAE level (data not shown), although the optimal f_2/f_1 (the peaks of these functions), did vary somewhat between ears. The exceptions to this, as mentioned above, were the individual f_2/f_1 functions at 24 kHz. Generally, the noise-floor levels did not vary greatly between animals, with the exception of one frequency range (Fig. 2, 4 kHz) at high frequency ratios.

At each value of f_2/f_1, the DPOAE levels were averaged across all subjects having complete ranges. The f_2/f_1 which elicited the strongest mean L_{DP} at each f_2 frequency and primary-tone level was considered the optimal f_2/f_1. There often were two or more f_2/f_1 ratios that elicited DPOAEs that were similar in strength. Such f_2/f_1 ratios were typically neighboring values, or

at most separated by a small dip. If there were one or more DPOAEs within 1.0 dB SPL of the strongest DPOAE for a particular stimulus condition, the mean of the associated f_2/f_1 ratios was considered the optimal f_2/f_1. This mean was, in most cases, very similar to the f_2/f_1 ratio which elicited the strongest DPOAEs. Using this method meant that a single value was chosen to represent optimal f_2/f_1 ratio for each combination of stimulus parameters (e.g., $L_1 = L_2 = 50$ dB SPL at 3 kHz, $L_1 = L_2 = 50$ dB SPL at 4 kHz, etc.). Accordingly, we were not able to determine statistical significance or effect size of sex or ear differences in the optimal f_2/f_1.

The mean optimal f_2/f_1 for each stimulus parameter ranged between 1.119 and 1.386 (Table 1). The mean optimal f_2/f_1 (averaged across all ears and all primary-tone levels) decreased with increasing frequency from 3 – 17 kHz, and then greatly increased for $f_2 = 24$ kHz (Fig. 3A). The mean optimal f_2/f_1 for individual primary-tone levels exhibited a similar trend as the average across all primary-tone levels (Fig. 3A). When examining ear differences, only animals in which both ears were tested were considered. The ear difference in the mean optimal f_2/f_1 was minimal at most frequencies (no difference at 24 kHz), and was largest at 3 kHz (right ear mean = 1.292; left ear mean = 1.383) (Fig. 3B). Sex differences in the mean optimal f_2/f_1 ratio were minimal at all f_2 frequencies (Fig. 3C). The mean optimal f_2/f_1 (across all frequencies and all ears) and the mean optimal f_2/f_1 for each individual f_2 frequency (across all ears) both increased with increasing primary-tone level (Fig. 4A, Table 1). The mean optimal f_2/f_1 (across all frequencies) was similar in both ears (Fig. 4B), and both sexes (Fig. 4C), especially with 62 and 68 dB SPL primary tones.

When all stimulus parameters and all subjects were considered (17 ears: 12 right, 5 left) the mean optimal f_2/f_1 was 1.224. For animals in which both ears were tested (n = 5), the mean optimal f_2/f_1 was 1.226. We have shown DPgrams in Fig. 5 to directly compare the effect of f_2/f_1 ratio over the limited range of 1.18 to 1.24 on the average L_{DP}. The DPOAEs elicited by each of these f_2/f_1 ratios were very similar in amplitude within each f_2 frequency, especially below 6 kHz.

The effect of decreasing L_2 below L_1

Mean I/O functions (across all ears) are shown in Fig. 6 for each primary-tone level difference ($L_1 - L_2 = 0$, 5, 10 dB SPL). In general, the L_{DP} increased as the primary-tone levels increased, and a maximum L_{DP} was elicited when $L_1 = 74$ dB SPL, the highest level tested. Figure 7 shows DPgrams for each L_1 and $L_1 - L_2$. These DPgrams show significant maxima in L_{DP} at 8.5 and 17 kHz at all primary-tone levels above 50 dB SPL. These peaks became more pronounced as primary-tone levels were increased. There was essentially no effect of decreasing L_2 below L_1 for most conditions, but for some conditions there was a small or, for even fewer conditions, a medium effect size. However, there was no consistent pattern to suggest that using a larger primary-tone level difference elicits stronger DPOAE levels in the ears of young, healthy marmosets.

Sex and ear differences

Using equilevel primary tones, the effects of sex (6 males, 6 females, right ear only) and ear (6 right ears, 6 left ears) on L_{DP} were determined (Fig. 8A–D). Female DPOAE levels were larger than those of the males, regardless of the primary-tone level (Fig. 8A) or f_2 frequency (Fig. 8B). With 62 dB SPL primary tones, the absolute difference in mean L_{DP} (mean female L_{DP} –mean male L_{DP}) ranged from 3.45 dB SPL (at 6 kHz) to 11.81 dB SPL (at 24 kHz). There was a medium effect size at 3, 8, and 12 kHz, and a large effect at 4, 6, 17, and 24 kHz (Fig 8B). The mean difference across frequencies (with $L_1 = L_2 = 62$ dB SPL) was 6.20 dB SPL. When averaged across all f_2 frequencies, the absolute difference in mean L_{DP} was highest at 50 dB SPL (10.27 dB SPL) and lowest at 74 dB SPL (3.15 dB SPL). The effect size was large

when $L_1 = L_2 = 50$ to 68 dB SPL, and small when $L_1 = L_2 = 74$ dB SPL (Fig. 8A). The mean difference across frequencies and all primary-tone levels was 5.84 dB SPL.

DPOAEs tended to be slightly stronger in the right ear than the left ear (Fig. 8C), regardless of the primary-tone level ($L_1 = L_2$), though the effect size was small, similar to humans. As a function of f_2 frequency, the mean L_{DP} was stronger in the right ear than the left at each frequency, except 24 kHz, where it is reversed (Fig. 8D). The effect size was small at 6, 8, 17, and 24 kHz, but was in the opposite direction at 24 kHz. The effect size was medium at 4 kHz, and was large at 3 and 12 kHz.

I/O Functions

To determine the slopes of individual I/O functions obtained with equilevel primary tones (mean functions are shown in Fig. 6), we fitted a linear-regression equation to the most linear portion of the functions (Mills & Rubel, 1996; Coro & Kössl, 2001; Meenderink & van Dijk, 2004). The most linear growth occurred between $L_1 = L_2 = 50$ to 68 dB SPL (Fig. 6), and the functions began to saturate at 74 dB SPL. The slopes (in dB/dB) increased from 3 kHz (0.536) to 12 kHz (1. 287), decreased at 17 kHz (0.887), and increased at 24 kHz (0.989).

Discussion

Frequency ratio (f_2/f_1) optimization

Stimulus parameters such as the frequency and level of the primary tones, frequency ratio, and primary-tone level difference all contribute to DPOAE amplitude (Wilson, 1980; Harris *et al.*, 1989; Brown & Gaskill, 1990a; Gaskill & Brown, 1990; Park *et al.*, 1995; Lasky *et al.*, 1995; Abdala, 1996; for a review, see Probst *et al.*, 1991). The primary goal of this study was to determine the optimal stimulus parameters for eliciting robust DPOAEs in this species.

Historically, many methods have been employed to study the effects of varying f_2/f_1 on DPOAE amplitude. One method involves fixing one of the primary-tone frequencies, either f_1 (Kim, 1980; Furst *et al.*, 1988; Brown & Gaskill, 1990a; Gaskill & Brown, 1990) or f_2 (Schmiedt, 1986; Lasky *et al.*, 1995; Park *et al.*, 1995; Dreisbach & Siegel, 2001) and systematically varying the second primary tone to satisfy a set of predetermined f_2/f_1 ratios. Other investigators have varied f_1 and f_2 to satisfy a set of predetermined f_2/f_1 ratios around a set of geometric mean frequencies (f_{GM}) of the primary tones ($f_{GM} = (f_1 \times f_2)0.5$) (Nielsen *et al.*, 1993) or a set of fixed DPOAE frequencies (Kim, 1980; Wilson, 1980; Horner *et al.*, 1985; Lonsbury-Martin *et al.*, 1987; Harris *et al.*, 1989; Smurzynski *et al.*, 1990). We have employed the fixed-f_2 method which has been used to optimize the f_2/f_1 ratio in both rhesus monkeys (Park *et al.*, 1995; Lasky *et al.*, 1995) and humans (Schmiedt, 1986; Lasky *et al.*, 1998; Dreisbach & Siegel, 2001). It is widely accepted that DPOAEs are first generated near the region where f_2 is registered, so by using the fixed-f_2 paradigm, we have attempted to ensure that any variation in DPOAE level is due solely to the effects of frequency ratio and not to the effect of changing the place of DPOAE generation (Moulin, 2000).

In humans, a number of studies have determined the optimal f_2/f_1, and the reported mean optimal f_2/f_1 (1.22 – 1.23) has been similar across studies, regardless of method (Harris *et al.*, 1989; Gaskill & Brown, 1990; Nielsen *et al.*, 1993). In fact, Nielsen *et al.* (1993) noted that robust DPOAEs could be elicited at all test frequencies with an f_2/f_1 between 1.21 – 1.25.

In non-human primates, DPOAE data so far have been reported only in the rhesus monkey (*Macaca mulatta*). Park *et al.* (1995) determined that the mean optimal f_2/f_1 for the rhesus monkey was 1.21 using a fixed-f_2 paradigm ($f_2 = 1 – 8$ kHz, $L_1 = L_2 = 55$ to 85 dB SPL). Lasky *et al.* (1995), also using the fixed-f_2 paradigm, measured DPOAEs at four frequencies between 1 and 12 kHz using equilevel primary tones of 55, 60, and 65 dB SPL, and reported a similar

optimal f_2/f_1 ranging from 1.175 – 1.225. Our data showed that strong DPOAEs could be elicited between 3 – 24 kHz with a frequency ratio between 1.18 – 1.24, and that the mean optimal f_2/f_1 ratio was 1.224. This agrees well with results from humans and Old World primates.

The mean optimal f_2/f_1 does not, however, elicit DPOAEs at maximum amplitude at all frequencies. Our results in the marmoset agreed with previous findings in humans (Wilson, 1980; Harris *et al.*, 1989; Whitehead *et al.*, 1992a; Bowman *et al.*, 2000) and rhesus monkeys (Park *et al.*, 1995); that the optimal f_2/f_1 increases with increasing primary-tone level. Additionally, in rabbits (Lonsbury-Martin *et al.*, 1987; Whitehead *et al.*, 1992a), bats (Kössl, 1994; Frank & Kössl, 1995), rhesus monkeys (Lasky *et al.*, 1995; Park *et al.*, 1995), and humans (Harris *et al.*, 1989; Abdala, 1996; Bowman *et al.*, 2000, Brown *et al.*, 2000), the optimal f_2/f_1 ratio decreases with increasing f_2 frequency. To our knowledge, there is only one study in humans in which the f_2/f_1 ratio has been optimized at frequencies greater than 9 kHz. Dreisbach and Siegel (2001) extended their test-frequency range to 13 kHz to determine if the same cochlear mechanics applied at higher frequencies. They showed that the optimal f_2/f_1 ratio continued to decrease through f_2 frequencies of 10 kHz (when the emission probe was placed 10 – 15 mm from the eardrum). However, there was an increase in the f_2/f_1 ratio yielding the maximum amplitude DPOAEs at 13 kHz. In the marmoset, the optimal f_2/f_1 ratio continued to decrease through 17 kHz, but then increased at 24 kHz. In humans, Bowman et al. (2000) reported small sex differences in the optimal f_2/f_1 that were not statistically significant. The marmoset data agree well with this study; the mean optimal f_2/f_1 ratios shown here for male and female marmosets were very similar across f_2 frequency and primary-tone level.

Between 6 – 17 kHz (and at low- to mid-primary-tone levels at 4 kHz) the marmoset DPOAE levels were lowest at narrow and wide frequency ratios and maximal at mid-f_2/f_1 ratios, exhibiting the characteristic bandpass shape that has been reported by other investigators (Wilson, 1980; Brown & Kemp, 1985; Lonsbury-Martin *et al.*, 1987; Harris *et al.*, 1989; Brown & Gaskill, 1990a, b; Gaskill & Brown, 1990, Whitehead *et al.*, 1992; Brown *et al.*, 1993; Stover *et al.*, 1994; Park *et al.*, 1995; Lasky *et al.*, 1995; Abdala, 1996; Dreisbach & Siegel, 2001). Some researchers have suggested that the characteristic bandpass shape of these functions may be due to a second filter inherent in the cochlea (Allen, 1990b; Brown & Gaskill; 1990b; Neely & Stover, 1993; Stover *et al.*, 1994). However, studies which have used models of the basilar membrane lacking a second filter suggest that this is not the case (Matthews & Molnar, 1986; Neely & Stover, 1997; Kanis & de Boer, 1997; van Hengel & Duifhuis, 2000). Fahey *et al.* (2006) hypothesized that vector cancellation of DPOAEs generated from different sources causes the f_2/f_1 functions to exhibit bandpass characteristics. They measured DPOAEs in humans using the fixed-f_2 paradigm, but f_1 was selected at frequencies which would minimize vector cancellation. Under these conditions the f_2/f_1 functions lost their bandpass shape, indicating that the characteristic decline in DPOAE amplitude on either side of the peak is primarily due to vector cancellations.

Studies in humans (Harris *et al.*, 1989; Brown & Gaskill, 1990a; Whitehead *et al.*, 1992; Stover *et al.*, 1992; Abdala, 1996; Dreisbach & Siegel, 2001) have shown that the f_2/f_1 functions become sharper (steeper slopes on either side of the peak) with increasing frequency at f_2 frequencies up to 4 – 13 kHz. Marmoset results agree well with these findings at f_2 frequencies between 3 and 8.5 kHz and at 17 kHz. It has been suggested that the frequency-dependent increase in sharpness (of the f_2/f_1 functions) is due to sharper mechanical tuning at the basal end of the basilar membrane (Dreisbach & Siegel, 2001). However, a study in rhesus monkeys (Lasky *et al.*, 1995) showed that at frequencies below 12 kHz, the f_2/f_1 functions became sharper as f_2 was increased, but at 12 kHz the f_2/f_1 function exhibited a less sharp peak. We have seen the same trend to (what appears to be) an even greater extent in marmosets at 12 kHz (Figs. 1, 2).

Data Omission

We have reported that all errors in calibrating the primary tones occurred at 12 and 24 kHz when the frequency separation was large. Due to these calibration errors, the entire range of f_2/f_1 ratios was not presented and therefore we omitted data from two ears (1 right, 1 left ear) in our analysis of the 12-kHz range and six ears (3 right, 3 left) in the 24-kHz range. While optimizing the f_2/f_1 ratio, we set stringent limits on the levels of the primary tones. They had to fall within 2.0 dB SPL of the target levels. Because it was easy to present 24-kHz tones to some ears and not others, we suspect that the problems we encountered at this frequency were due to the individual transfer functions of the subject's ear canals. In these cases the data for the entire frequency range were excluded from analysis. These limits also suggest that the data we were able to collect at 12 and 24 kHz were reliable.

The effect of decreasing L_2 below L_1

Using the paradigm of holding L_1 constant between $50 - 74$ dB SPL and varying L_2 such that $L_1 - L_2 = 0, 5,$ and 10 dB SPL, we have reported that increasing $L_1 - L_2$ does not significantly increase DPOAE levels between 3 and 24 kHz in marmosets. This agrees very well with the results in rhesus monkeys (Park *et al.*, 1995; Lasky *et al.*, 1995) and humans (Gaskill & Brown, 1990; Whitehead *et al.*, 1995). These human studies showed that DPOAE level is more dependent upon L_1 than L_2. Additionally, Whitehead *et al.* (1995) showed that the relative levels of primary tones (when $L_1 - L_2$ 10 dB) only slightly affect the amplitude of DPOAEs in healthy ears.

Normative DPOAEs

The slopes of the marmoset I/O functions are similar to the results of Lasky *et al.* (1995), who reported an increase in slope with increasing frequency in both humans and rhesus monkeys, with the slopes of the I/O functions between $3 - 4$ kHz being approximately 1.0. The slopes of the marmoset I/O functions at 3, 4, 17 and 24 kHz were below 1.0 (compressive power functions – the level of the DPOAE increased at a slower rate than the level of the primary tones), and at 6 and 8 kHz the slopes were greater than 1.0 (expansive power functions – the level of the DPOAE increased at a faster rate than the level of the primary tones). Lasky *et al.* (1995) measured DPOAEs at frequencies up to 20 kHz and reported a general increase in the slopes of the I/O functions through 20 kHz, although there were non-monotonicities throughout the function (slope vs. frequency) where the slope decreased at 16 kHz and increased again at 24 kHz. The non-monotonicities observed in the marmoset slope function followed a similar trend, though the f_2 frequencies showing inflections were not identical to Lasky's (16 vs 17 kHz and 20 vs 24 kHz). The differences observed in the slopes of the I/O functions in marmoset vs. rhesus monkey may be due to the methods used to determine the slopes of these functions. It is unclear to us which data points were used by Lasky's group to determine the slopes of the I/O functions.

Park *et al.* (1995) found that the average DPOAE amplitude in six rhesus monkeys was $55 - 70$ dB below the (equilevel) primary tones, depending on primary-tone level and frequency. McFadden *et al.* (2006a) showed that, on average, rhesus monkey DPOAEs were approximately $45 - 55$ dB SPL below the (equilevel) primary tones. Lasky *et al.* (1995) reported average rhesus DPOAE levels to be stronger than human DPOAEs by $10 - 15$ dB between $f_2 = 3 - 10$ kHz (and less around $f_2 = 1$ kHz). The mean DPOAE levels of the marmoset ranged from 50 to 60 dB SPL below the levels of the primary tones. These DPOAE levels are not equivalent to those of humans, which are approximately 60 dB SPL below the level of the primary tones (for a review, see Probst *et al.*, 1991). These differences may be explained by size differences in the ear canals or by the effects of ketamine, which has been shown to increase DPOAE levels in mice (Harel *et al.*, 1997).

The sex differences in the normative DPOAE level are in the same direction as has been reported in humans (e.g. Dunckley & Dreisbach, 2004), and other animals (e.g. frogs: Vassilakis *et al*., 2004; rhesus monkeys: McFadden et al., 2006a). These studies have shown that the mean L_{DP} is slightly stronger in females than males. The sex differences we have reported seem to be the largest reported in primates thus far. In humans, Dunckley & Dreisbach (2004) investigated sex differences in L_{DP} (with $L_1 = 60$ dB SPL, $L_2 = 45$, and $f_2/f_1 = 1.22$) and showed that the absolute difference in the mean L_{DP} (mean female L_{DP} − mean male L_{DP}) ranged from -5.46 dB SPL (at 9 kHz) to 6.07 dB SPL (at 13 kHz) and the mean difference in L_{DP} was reported to be 2.08 dB SPL. In marmosets, the absolute difference in mean L_{DP}, with 62 dB SPL (equilevel) tones, ranged from 3.14 – 11.8 dB SPL (mean = 6.20 dB SPL). The sex difference in marmoset DPOAEs was even more pronounced when $L_1 - L_2$ was 10 dB SPL ($L_1 = 62$ dB SPL), ranging from 4.32 – 15.48 dB SPL (mean = 7.60 dB SPL) (data not shown). The effect size for sex differences in rhesus monkeys was dependent on the frequency range and ear (McFadden *et al*., 2006a). In the right ear, the effect sizes (mean female L_{DP} − mean male L_{DP}) were -0.28, 0.17, and 0.02 for frequency ranges of 2.0 – 2.5, 3.0 – 3.5, and 5.0 – 5.5 kHz, respectively. In marmosets, the effect size for sex differences were much larger, ranging from 0.51 to 2.05 (with $L_1 = L_2 = 62$ dB SPL) as a function of frequency and from 0.37 to 1.22 as a function of primary-tone level (averaged across all f_2 frequencies).

Conclusions

Strong DPOAEs were easily elicited in the common marmoset. The effect of varying stimulus parameters was similar to the effects reported in humans and rhesus monkeys. The mean optimal f_2/f_1 ratio was 1.22, but f_2/f_1 ratios between 1.18 – 1.24 elicited DPOAEs that were similar in strength. The mean optimal f_2/f_1 was slightly dependent on primary-tone level and strongly dependent upon f_2 frequency. No systematic change in DPOAE amplitude was observed in the ears of these young, healthy marmosets when decreasing L_2 below L_1 by 5 or 10 dB SPL. The sex difference in DPOAE levels we reported is the largest observed in primates, including humans.

The data presented here suggest that the marmoset may be an excellent model to study the mechanisms underlying sex differences in otoacoustic emissions, but these differences should be further examined with CEOAEs, which typically exhibit much larger differences between sexes than DPOAEs (e.g. McFadden et al., 2006a). In future studies we will examine the effects of age and sex on normative DPOAEs and CEOAEs, with and without contralateral stimulation. To date, no SOAEs have been observed in marmosets, but the search will continue.

Acknowledgements

Abbreviations

OAE

    otoacoustic emission

f_1, L_1

frequency and level of lower-frequency primary tone

f$_2$, L$_2$

frequency and level of higher-frequency primary tone

f$_2$/f$_1$

frequency ratio

L$_1$ − L$_2$

primary-tone level difference

L$_{DP}$

DPOAE level

f$_{DP}$

DPOAE frequency

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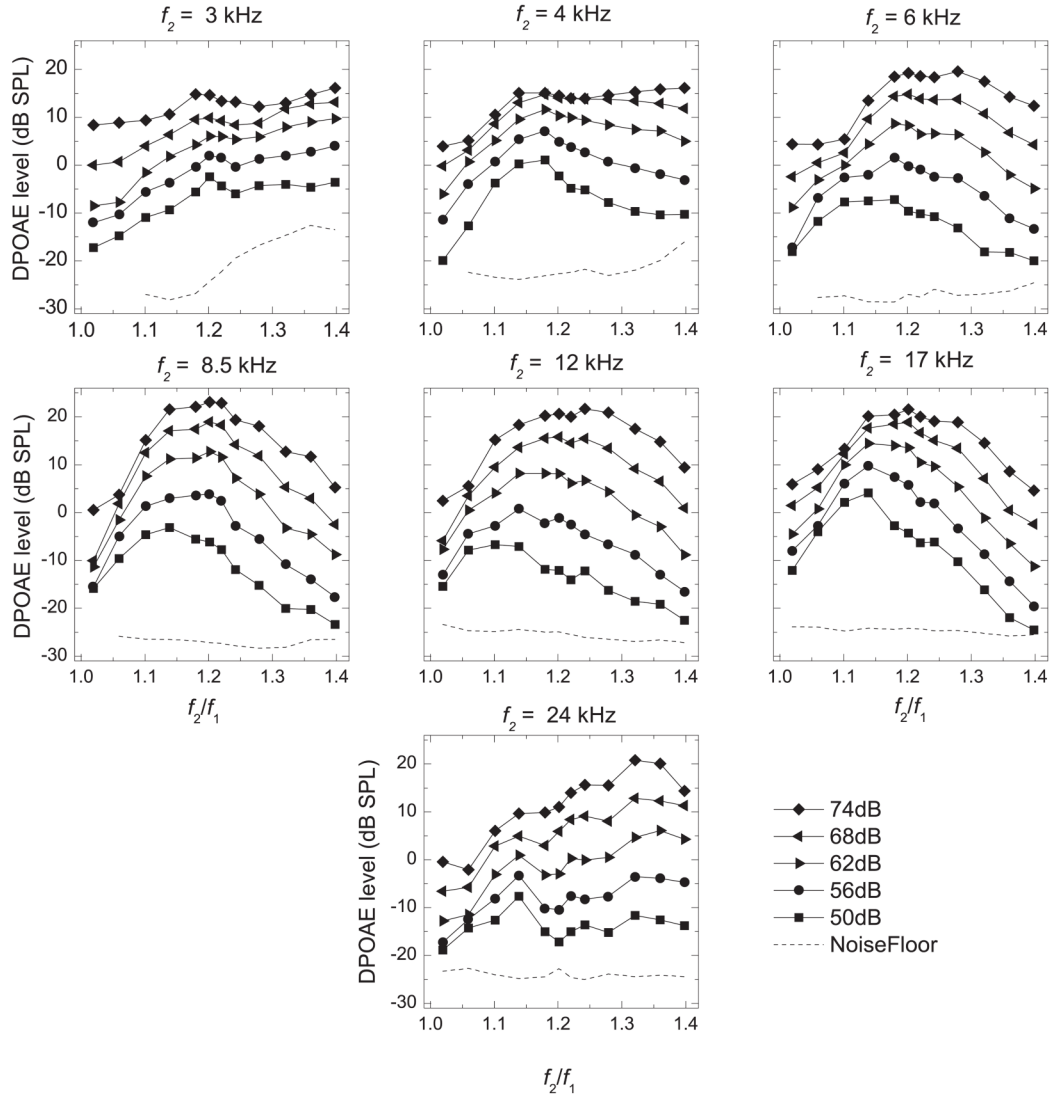
Figure 1.
Frequency-ratio (f_2/f_1) functions showing L_{DP} (ordinate) vs. f_2/f_1 (abscissa) for each f_2 frequency (f_2 is specified above the individual panels). DPOAEs were obtained with equilevel primaries from 50 – 74 dB SPL (in 6-dB increments) as f_2/f_1 was increased from 1.02 – 1.40 in 0.04 increments (0.02 increments between 1.18 and 1.24). Responses were averaged across all ears (n = 17).
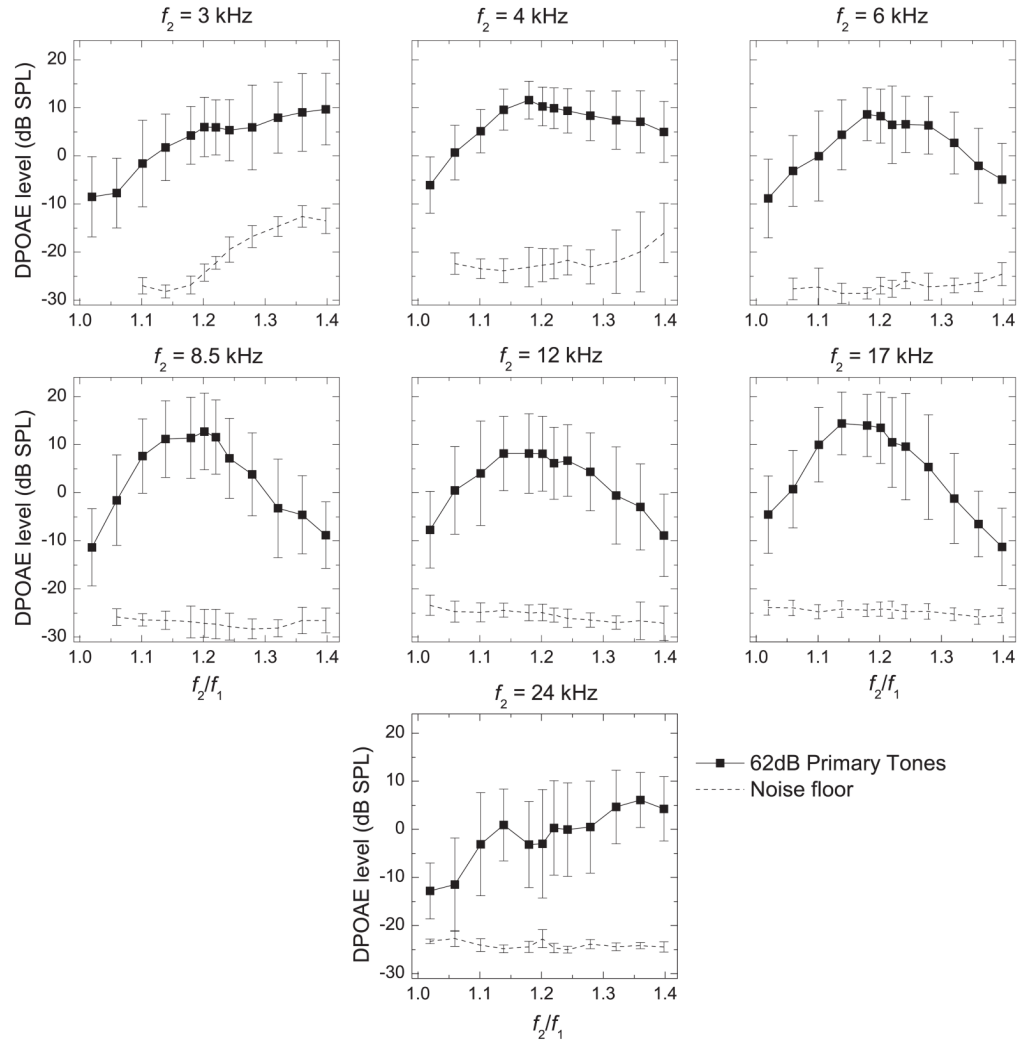
Figure 2.
Frequency-ratio (f_2/f_1) functions for each f_2 frequency (axes as in Fig. 1). The DPOAEs shown here were obtained using equilevel primary tones at 62 dB SPL and were averaged across all animals and ears. Error bars are shown to demonstrate the typical amount of inter-subject and inter-ear variability observed in the DPOAE levels and the noise floor.
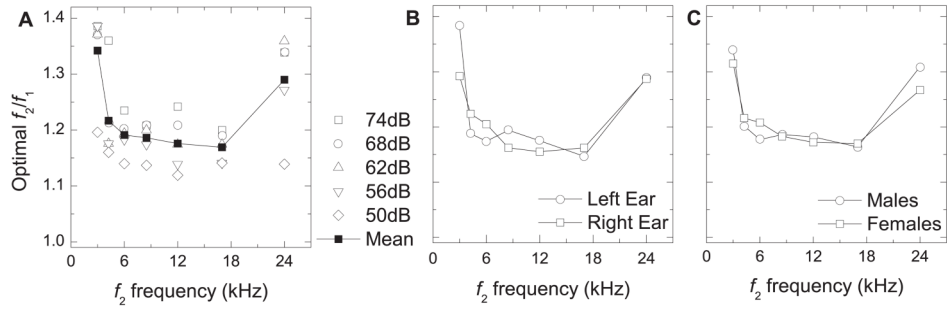
Figure 3.
Mean optimal f_2/f_1 ratio (ordinate) vs. f_2 frequency (abscissa). **A)** The f_2/f_1 ratios which elicited the most robust mean DPOAEs (averaged across all ears) were considered as the mean optimal f_2/f_1 ratios. **B)** Right vs. left ear. DPOAEs were averaged only in subjects in which both ears were tested and the mean optimal f_2/f_1 was that which elicited the most robust mean DPOAE (n = 5). **C)** Males vs. females. DPOAEs were averaged across both ears in males and females and the f_2/f_1 which elicited the most robust mean DPOAE was considered optimal.
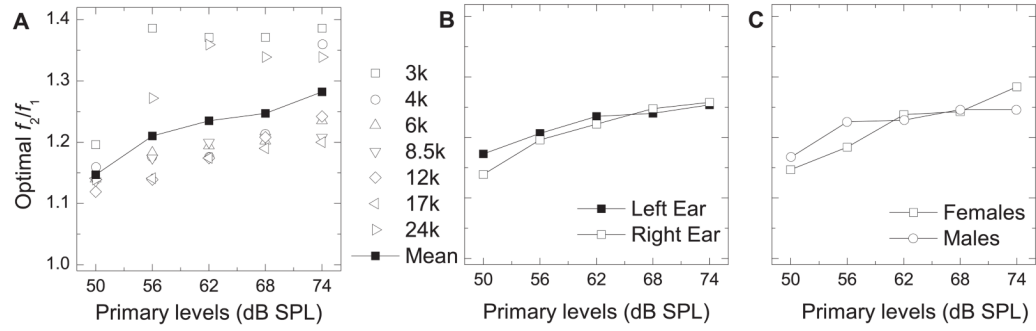
Figure 4.
Mean optimal f_2/f_1 ratio (ordinate) vs. primary-tone level (abscissa). **A)** The f_2/f_1 ratios which elicited the most robust DPOAEs for each ear were averaged to determine the mean optimal f_2/f_1. **B)** Right vs. left ear. DPOAEs were averaged only in subjects in which both ears were tested and the mean optimal f_2/f_1 was that which elicited the most robust mean DPOAE (n = 10 ears). **C)** Male vs. female. DPOAEs were averaged across both ears in males and females and the f_2/f_1 which elicited the most robust mean DPOAE was considered optimal.
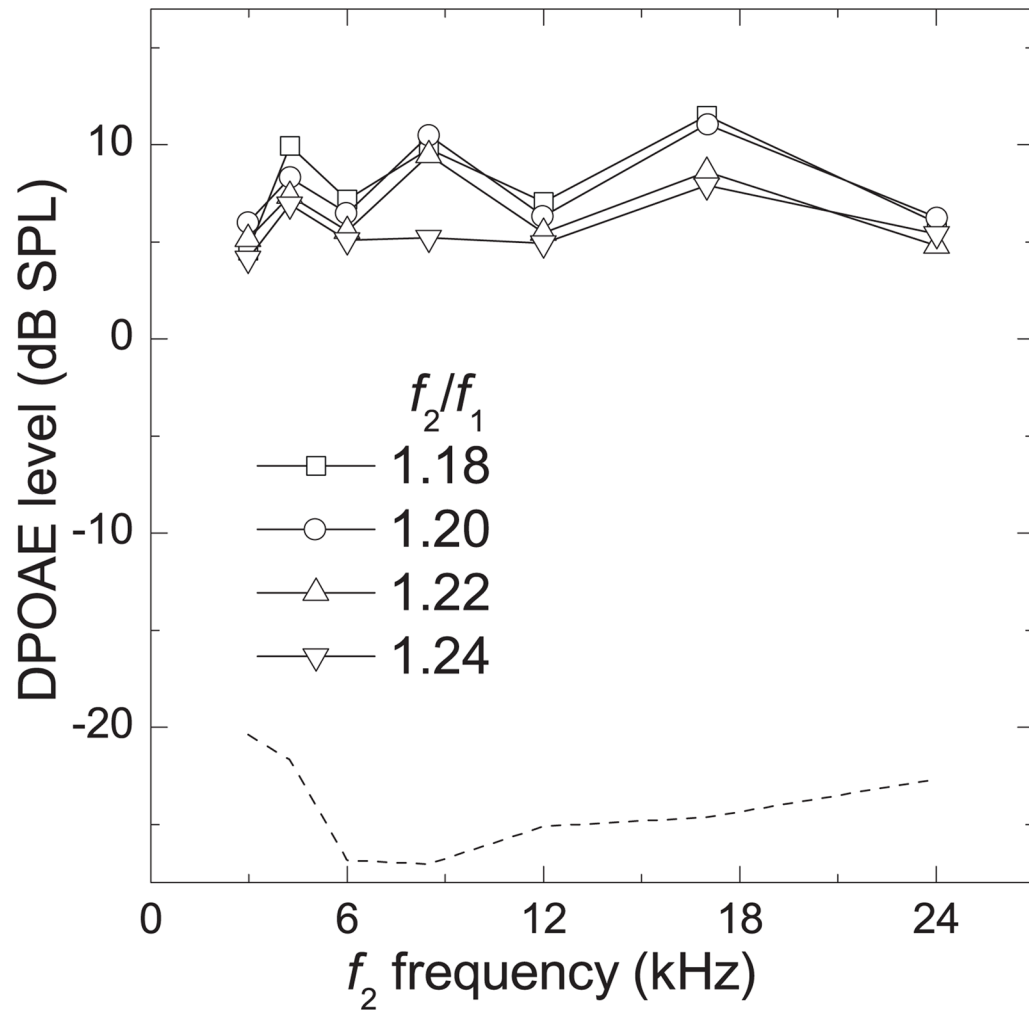
Figure 5.
DPgram (L_{DP} vs. f_2 frequency) for f_2/f_1 values of 1.18 – 1.24. DPOAE levels were averaged across all ears (n = 17). Dashed line represents average noise floor.
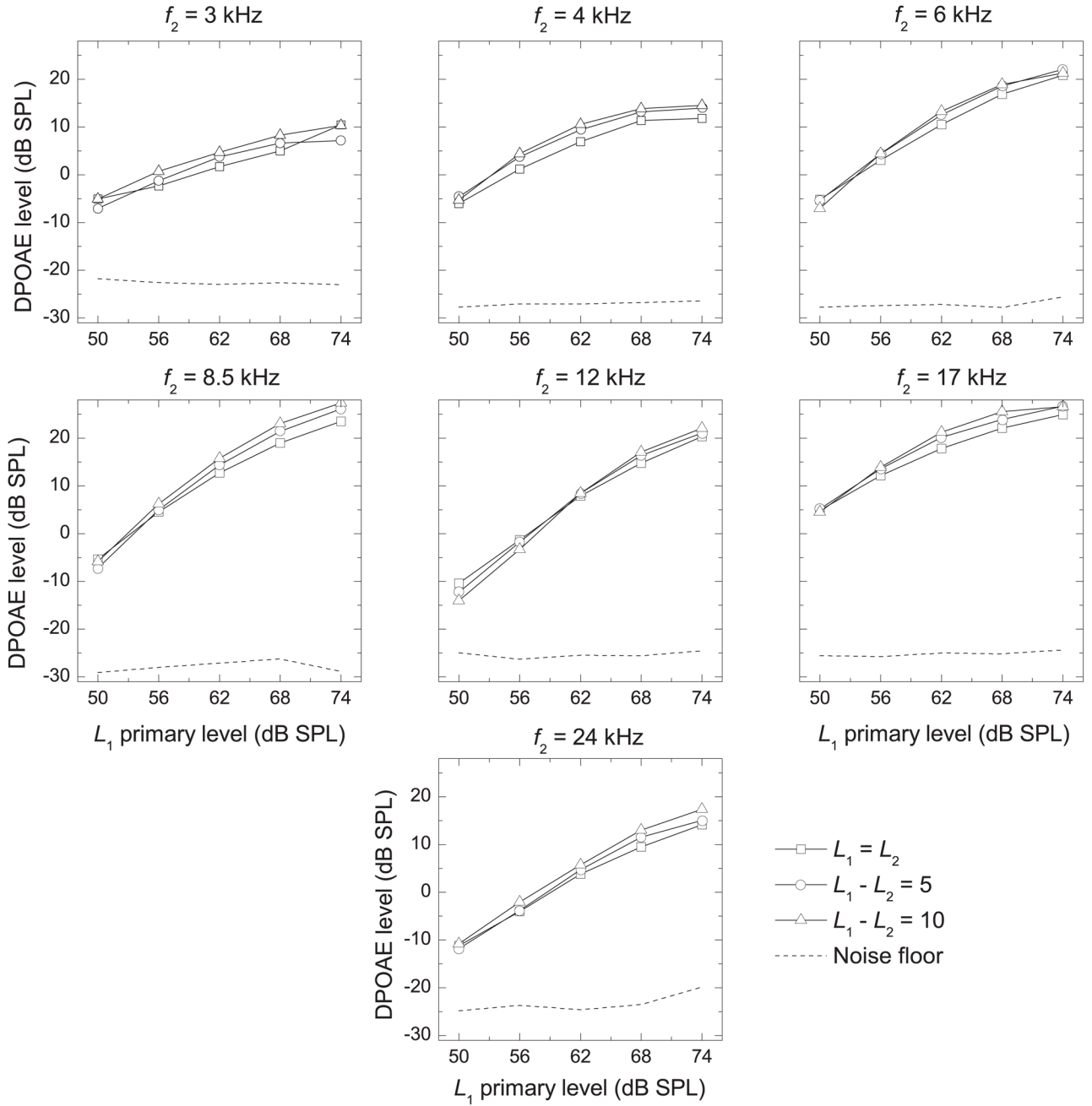
Figure 6.
I/O Functions (L_{DP} vs. L_1 Primary Tone) at each f_2 frequency (f_2 indicated above the panel) for each primary-tone level difference. DPOAE levels were averaged across all ears (n = 18).
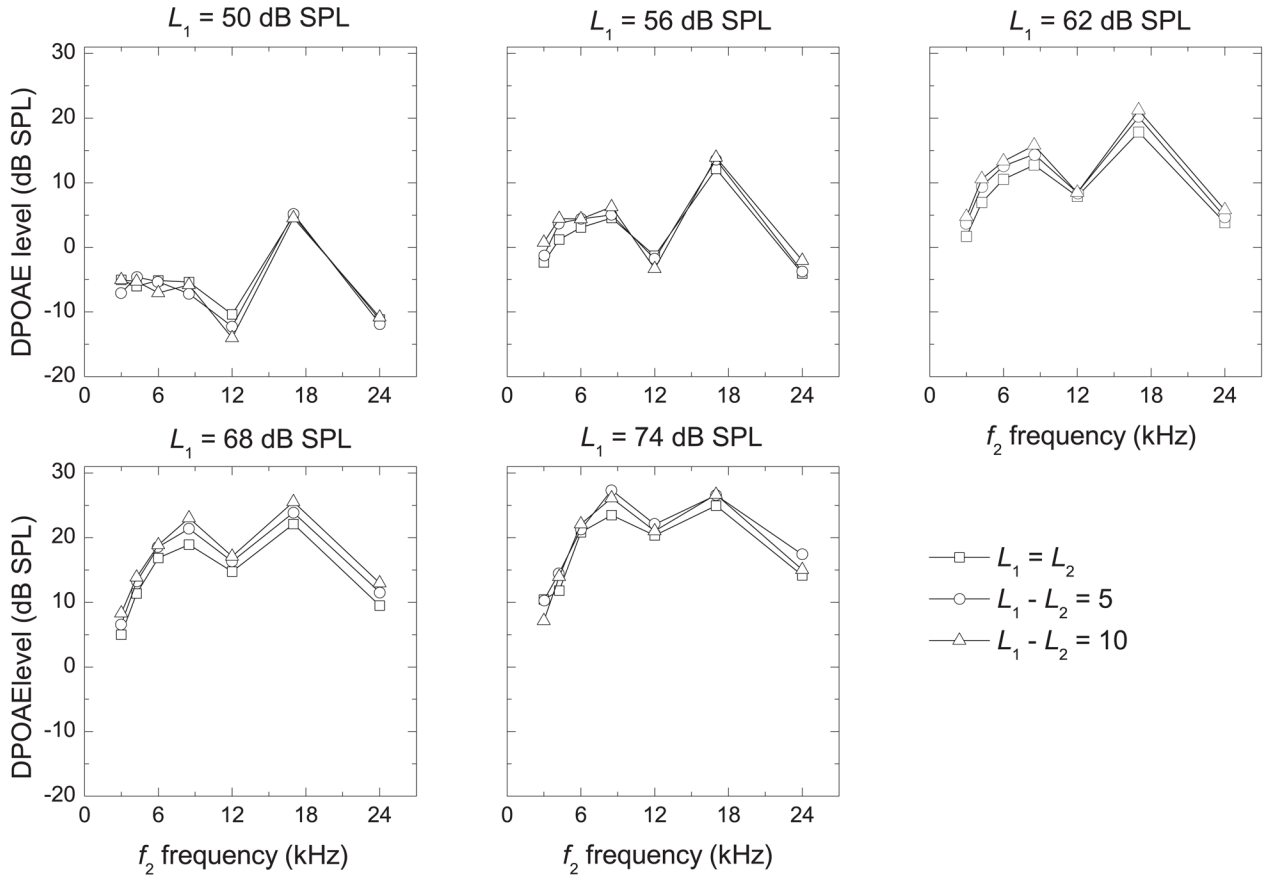
Figure 7.
DPgrams (L_{DP} vs. f_2 frequency) at each primary-tone level for each difference in primary-tone level. DPOAE levels were averaged across all ears (n = 18).
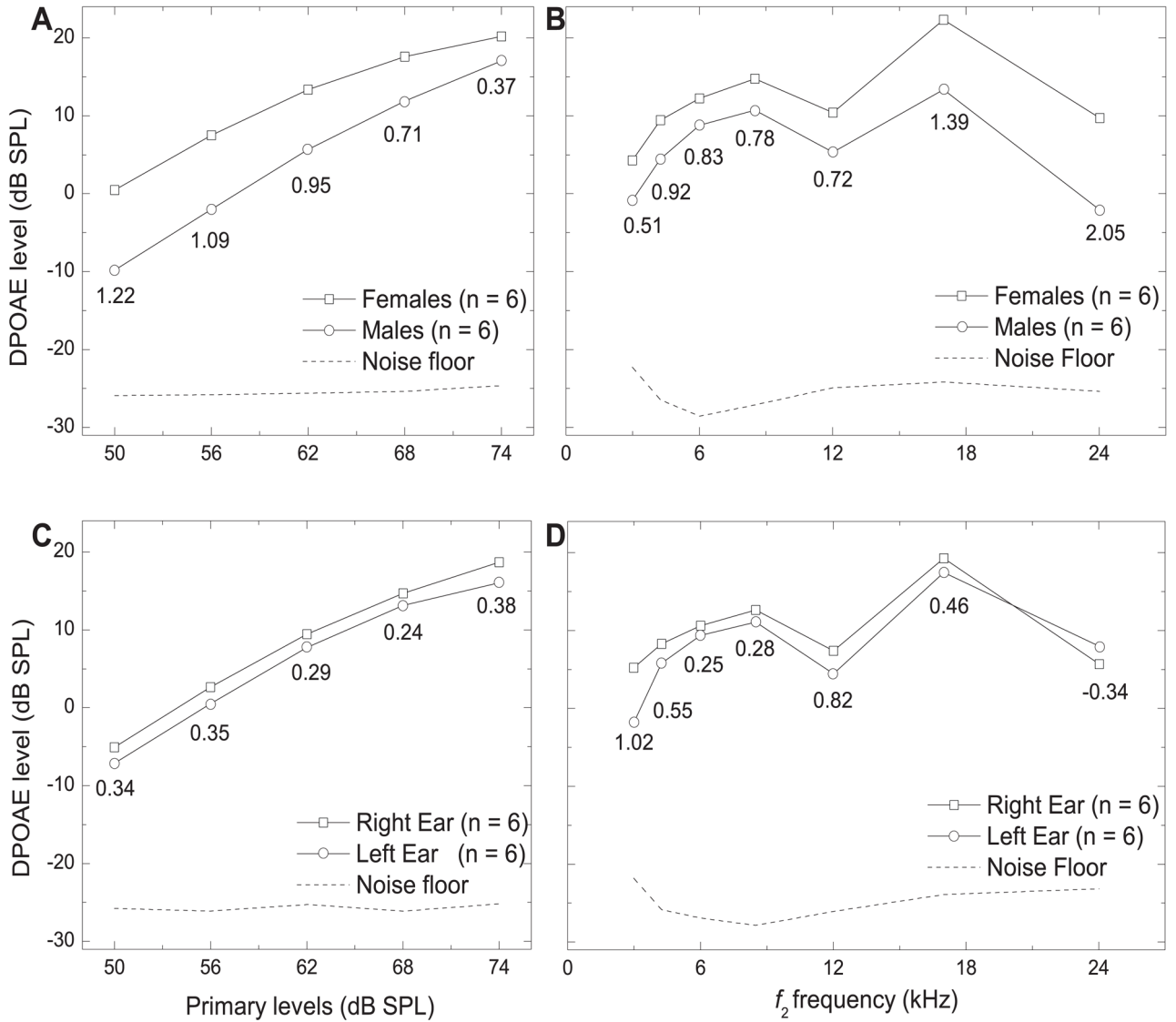
Figure 8.
A) I/O Functions (L_{DP} vs. L_1 Primary Tone) for males vs. females (data was averaged across all f_2 frequencies). **B)** DPgrams (L_{DP} vs. f_2 frequency) for males vs. females ($L_1 = L_2 = 62$ dB SPL). **C)** I/O Functions (L_{DP} vs. L_1 Primary Tone) for right vs. left ear (data was averaged across all f_2 frequencies). **D)** DPgrams (L_{DP} vs. f_2 frequency) for right vs. left ear. Effect sizes are shown below the lower of the two compared mean L_{DP} values.
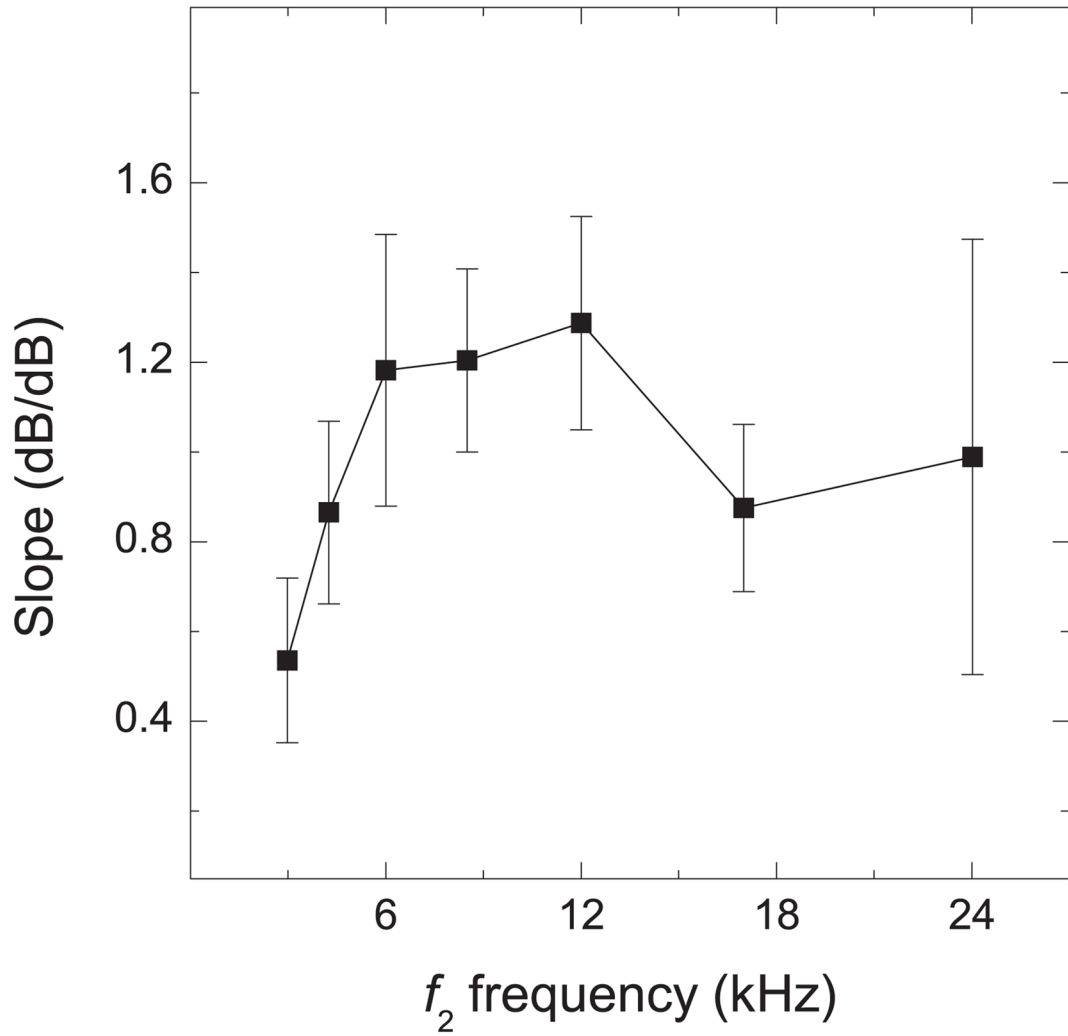
Figure 9.
Slope Functions (Slope vs. f_2 frequency). The slopes of the linear-regression equations characterizing the I/O functions from 50 – 68 dB SPL (averaged across all ears of both sexes). Error bars are shown to indicate the amount of inter-subject and inter-ear variability in the slope of the I/O functions.

Table 1

Mean optimal f_2/f_1 ratio for each combination of stimulus parameters.

	f₂ (Hz)							
	2978	**4248**	**6005**	**8496**	**12,011**	**16,992**	**24,023**	**Average**
L₁ = L₂ (db SPL)	*Optimal frequency ratio all animals (both ears)*							
50	1.196	1.160	1.140	1.137	1.119	1.141	1.139	1.147
56	1.386	1.176	1.183	1.174	1.139	1.141	1.272	1.210
62	1.371	1.176	1.194	1.200	1.174	1.174	1.359	1.235
68	1.371	1.213	1.202	1.208	1.208	1.190	1.339	1.247
74	1.386	1.360	1.235	1.208	1.242	1.200	1.339	1.282
Average	1.342	1.217	1.191	1.186	1.176	1.169	1.290	1.224