An experimental study of tympanic-membrane and manubrium vibrations in rats

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Abstract

Rats are potentially very useful for use in auditory research because the middle-ear structures are easily approachable and because rats are relatively inexpensive. The goal of the present study is to better characterize the mechanics of the rat middle ear by measuring frequency responses at multiple points on the tympanic membrane and manubrium. A laser Doppler vibrometer was used to measure the vibrations. Measurements were made on seven rats. Tympanic-membrane vibrations are presented for seven different points in the frequency range of 1 to 10 kHz. The repeatability of the measurements and the inter-animal variability at the umbo are presented. The vibration modes of the tympanic membrane and manubrium are also investigated.

Keywords: middle ear, rat, laser doppler vibrometry, manubrium, umbo

Introduction

It was suggested by Hellström et al. [1982] that the rat is of value in otological research because the middle-ear structures are easily approachable and because rats are less expensive than other species used in middle-ear research. Since almost all human genes known to be associated with disease have orthologues in the rat genome, and since rats have recently been added to the list of species whose genomes have been mapped [Rat Genome Sequencing Consortium, 2004], the rat could become an even more valuable tool in middle-ear research.

The literature contains few studies on rat middle-ear mechanics. Early studies by Beccari and Molinengo [1958] and by Ishii et al. [1964] reported the frequencies of maximum sensitivity of the ear, but no frequency responses were shown. More recent studies by Doan et al. [1996] and Bigelow et al. [1996, 1998] did provide frequency responses but they measured the frequency responses at only one point on the tympanic membrane. To fully understand the vibrations of the middle ear, one needs to measure displacements at multiple points.
The goal of the present study is to better characterize the mechanics of the rat middle ear by measuring frequency responses at multiple points.

Comparative anatomy

The middle ear of the rat contains all the anatomical structures found in the human middle ear. As one might suspect, the ossicles are much smaller in the rat than in humans; they are approximately one quarter as long [Judkins and Li, 1997]. The middle-ear morphology of the rat was described by Fleischer [1978] as exhibiting the *microtype* organization. This design has two distinctive characteristics: (1) the malleus is fused to the tympanic ring at the gonial bone; and (2) there is a large mass called the *orbicular apophysis* on the head of the malleus (Figure 1).

In humans, the area of the tympanic membrane is ~66 mm^2 [Donaldson et al., 1992], whereas in the rat it is only ~11 mm^2 [Zimmer et al., 1994]. The relative sizes of the pars tensa and pars flaccida are also quite different – while the human has a very small pars flaccida compared with the total size of the tympanic membrane, in rats the pars flaccida occupies between one quarter and one third of the tympanic-membrane area.

In humans, the malleus-incus complex is commonly viewed as having a rotational axis which, at least at low frequencies, runs through two suspensory ligaments: the posterior incudal ligament and the anterior mallear ligament. According to Fleischer [1978], despite the apparent fixation of the malleus to the tympanic ring in the microtype ear, the complex can still rotate. This connection and the anchoring of the short process of the incus form a rotational axis similar to that in humans (Figure 1). Rats and humans are, however, dissimilar in that the rat manubrium is almost parallel to the axis of rotation, which is not the case in humans. Fleischer [1978] also found, through experiments with an enlarged mechanical model, that the additional mass contributed by the presence of the orbicular apophysis shifts the centre of mass of the malleus-incus complex. This adds a second axis of rotation at high frequencies that runs along the transverse part of the malleus (Figure 1). He therefore concluded that microtype ears have two axes of rotation and two clearly defined modes of vibration of the malleus.

Materials and Methods

Specimen preparation

The measurements were carried out on seven Sprague Dawley rats supplied by Charles-River (St-Constant, Québec). Measurements were made within three hours of death. Table 1 provides details about the rats used in this study. The external ear was completely removed up to the cartilaginous part of the ear canal, and parts of the bony ear-canal wall were removed with a drill to optimize access to the tympanic membrane and manubrium. The air pressures on both sides of the tympanic membrane need to be equal for it to vibrate normally. To ensure this was the case, we drilled a small hole into the bulla, equalizing the pressures on the two sides of the tympanic membrane. Figure 2 illustrates the location of the tympanic bulla in the rat skull.

During the dissections, the area of interest was kept moist with saline solution to prevent the tympanic membrane and middle-ear structures from drying out. Once measurements were started, no more solution was applied to the middle ear. The area was sealed with Plasticine™ in order to avoid drying of the middle-ear structures.

Several studies have shown that the middle ear can remain relatively normal for hours, or even days, after death if special steps are taken to maintain the normal behaviour. Rosowski [1990] measured the input impedance in fresh and thawed human temporal bones and compared the measurements with *in vivo* data obtained with similar instruments. His measurements of *post mortem* middle ears compared well with the normal ranges for living ears. Measurements reported by Rosowski [1990] in guinea pigs and by Khanna and Tonndorf [1972] in the cat also suggest that the *post mortem* middle ear functions similarly to that of a live subject. Goode et al. [1993, 1996] compared umbo-motion measurements that they and others had made on cadavers with those made on live ears. In 1993, they concluded that the acoustic properties of the tympanic membrane were no different in live human ears and temporal bone models at low and mid frequencies. In 1996 they compared their own averaged results and showed that no significant differences exist below 6 kHz.

Acoustical system

An ER-2 Tubephone™ (Etymōtic Research) was used as the sound-delivery system, and the sound-pressure level was monitored with an ER-7C (Etymōtic Research) probe-microphone system. The sound-delivery and probe-microphone tubes were inserted into a 0.5-cc cylindrical sound chamber, sealed at the top with a glass cover slip. The tip of the probe-microphone tube was placed 2–3 mm from the eardrum. Measurements were made in a double-walled audiometric sound room (model C-14, Génie Audio, Québec).

The tympanic membranes were subjected to a slowly sweeping sine signal (0.25–10 kHz) at 60 dB SPL. Each sweep response took approximately 1 min to acquire after being averaged 30 times.

Optical system

Displacements were measured with a laser Doppler system (Polytec HLV 1000) coupled to an operating microscope (Zeiss, OPMI-1). A laser Doppler vibrometer makes use of the Doppler effect to measure the vibration velocity of the object of interest. Low-frequency noise is a problem with this technique because of the inherently low velocities at low vibration frequencies.

Laser vibrometry requires a sufficient amount of back-scattered light relative to the incident light beam. Given the high anisotropy coefficients for biological tissues (between 0.9 and 0.99) [Vogel et al., 1996], much light is lost to forward scattering. This was compensated for by placement of hollow glass micro beads (90–150 μm in diameter, Sigma) on the tympanic membrane. The micro beads were attached by simple capillary force. Given the presumably negligible mass of these beads, we were able to increase signal-to-noise ratio without affecting the frequency response.

Results

Linearity

We tested the system for linearity by measuring the displacement of the short process as a function of varying sound-pressure levels at 1, 2, 4 and 7 kHz. The fitting of straight lines through the data produced slopes close to 1 (1.01, 1.04, 0.98 and 1.01) and goodness of fit (R^2) values between 0.99 and 1, indicating linearity.

Noise floor

Measurement of the noise level on the canal wall in one animal indicated that the displacements at the umbo were at least 40 dB above the noise floor at most frequencies greater than 2.5 kHz in most animals, and more than 20 dB above the noise floor at frequencies greater than 1.5 kHz in all animals except rat 2, for which the response exhibited a sharp drop in amplitude at approximately 1.7 kHz. A combination of inherently small displacements at low frequencies and a higher noise-floor level at lower frequencies limited our ability to accurately measure displacements at frequencies below 1 kHz. For this reason, results below 1 kHz will be omitted.

Umbo vibrations

There was considerable variability in the umbo-vibration amplitudes from animal to animal. The range of displacements across animals was ~40 dB from 1−2 kHz and ~20 dB from 2–10 kHz (Figure 3). At ~2.5 kHz, the range was only ~6 dB.

The frequency responses measured in the previous studies by Bigelow et al. [1996] and Doan et al. [1996, 1998] were described as exhibiting a peak in the range 1.5–2.5 kHz and a second peak in the range 5–7.5 kHz (as well as a third peak beyond the frequency range that we measured). Our results can be described similarly, although in some cases the peaks are at somewhat lower or higher frequencies or are not well defined.

Figure 4 shows the results from our seven rats plotted with those of the five rats of Bigelow et al. [1996]. The amplitudes are all similar to those of Bigelow et al. [1996] between 3 and 10 kHz. For five of our rats, velocities at frequencies lower than ~3 kHz are much lower than those of Bigelow et al. The other two rats (rats 4 and 7) have responses similar to those of Bigelow et al. throughout the frequency range.

Manubrial vibrations

Displacements were measured at three points on the manubrium in all seven rats (and also at four points on the eardrum in three rats). The approximate measurement locations are shown in Figure 5. Measurements at all points were taken from the same viewing angle.

In rats 1, 2, 4 and 6 (Figure 6), the manubrial displacements were consistently largest at the umbo and smallest at the short process. In these rats, the shapes of the frequency responses were quite similar at all three points on the manubrium.

In rats 3, 5 and 7 (Figure 7), however, the patterns were quite different. In rat 3, the short-process displacements were still the smallest, but the mid-manubrium displacement was about the same as the umbo displacement at low frequencies and actually slightly larger than the umbo displacement at higher frequencies. In rat 5, the displacements were quite similar at all three points up to ~9 kHz, beyond which the short-process displacement became the largest. In rat 7, the pattern was very irregular.

The ratio of short-process displacement to umbo displacement was close to 1 in rats 3, 5 and 6: 0.75, 1.0 and 0.8, respectively. This is consistent with an axis of rotation which is more or less parallel to the manubrium. The ratio was significantly smaller, however, in rats 1, 2 and 4: 0.65, 0.5 and 0.3–0.6, respectively. In rat 7, the ratio varied dramatically with frequency.

Eardrum vibrations

Displacements were measured at four points on the eardrum in rats 1, 2 and 3 (Figure 8). The frequency-response shapes on the eardrum tended to be similar to one another and to that at the umbo, and the displacements tended to be largest in the posterior region and smallest at the umbo, but there were exceptions to these observations in each rat. For rat 1, the infero-anterior measurement begins to behave differently from the other three points above ~6 kHz. In the responses for rat 2, the infero-posterior measurement begins to behave differently at ~7 kHz. For rat 3, the four responses are similar up to ~6 kHz. The similarly shaped frequency responses at the four points on each pars tensa show that the vibration patterns are rather simple at these frequencies.

Repeatability

Repeat measurements from four rats are presented in order to examine the stability of the responses. Frequency responses at the middle of the manubrium taken over a period of 74 minutes in rat 4 are shown in Figure 9a. The displacement curves do not show large variations over the 74-minute period, remaining within +4 and –6 dB of the original measurement

throughout the frequency range. The time course is shown in Figure 9b, where we can see a drop in amplitude after 10 minutes at low frequencies; the response begins to stabilize after 30 minutes. In contrast, at higher frequencies the amplitude rises initially before dropping and becoming relatively stable.

The frequency responses taken at the middle of the manubrium for rat 5 maintained a similar shape for approximately one hour (Figure 10a). There was an overall shift downward in response and the higher-frequency peak drifted from ~7.3 kHz to ~8 kHz. The measurement taken after one minute practically overlaps the original one, showing amplitude differences of less than 0.5 dB over most of the frequency range. The subsequent measurements remained within –4 and +6 dB, except below 1.5 kHz where the signals were very noisy. Responses measured at 12 and 40 minutes are similar to one another, as are the ones at 30 and 54 minutes. Figure 10b shows that the amplitudes at low frequencies drop initially, whereas there is a rise in amplitude at higher frequencies. There is a steep rise in amplitude at all frequencies between 30 and 40 minutes, followed by a drop in amplitude between 40 and 54 minutes.

Repeat measurements taken at the short process of rat 6 are shown in Figure 11a. The frequency response maintained a similar shape for approximately one hour, although the displacements at the higher frequencies experienced changes in magnitude of up to 8 dB. A measurement taken one minute after the first one shows practically no deviations in amplitude over the frequency range. As we can see in Figure 11b, the system stabilizes at some point after the first 21 minutes, with responses thereafter remaining within 1 dB of each other at all frequencies. The responses at 2 kHz remain fairly constant throughout the entire 54 minutes.

Repeated measurements at the umbo in rat 7 are presented in Figure 12a. The responses are similar below 5 kHz, but behave erratically above that. A measurement taken 2 minutes after the original one already shows changes in the range of 6 dB above 6 kHz. The system is very stable up to 18 minutes at frequencies up to 5.5 kHz. This is also evident from Figure 12b where we see that the responses at 2 and 5 kHz do not exhibit large variations before the 18-minute mark, whereas the amplitude drops sharply at 8 kHz within the first 2 minutes.

At frequencies up to ~5 kHz, the measured displacements generally dropped during the first half hour and were fairly repeatable thereafter. The pattern was less regular at higher frequencies. The time-related changes in frequency response can probably be attributed in part to a drying out of the tympanic membrane, ligaments of the ossicular chain and middle-ear muscles in all animals.

Discussion

This study is the first to present measurements of rat tympanic-membrane and manubrium vibrations at points other than the umbo. Furthermore, the use of the sweeping-sinusoid stimulus allowed us to obtain a higher frequency resolution than in previous studies.

As mentioned earlier, Fleischer hypothesized that there are two axes of rotation in microtype ears: a low-frequency axis approximately parallel to the manubrium, and a high-frequency axis approximately perpendicular to the manubrium. Saunders and Summers [1982] measured vibrations at the short process and umbo in the mouse. They found, consistent with Fleischer's hypothesis, that at low frequencies the short-process and umbo displacements were similar, with a ratio of ~0.8. By ~10 kHz the ratio was becoming smaller and by ~18 kHz it had dropped below 0.5, consistent with a transition to Fleischer's high-frequency axis. Since the rat ear is larger than that of the mouse, one might expect the transition between modes to occur at a lower frequency in the rat.

Our measurements show short-process/umbo ratios of 0.75 and higher in three rats, consistent with the low-frequency findings of Saunders and Summers in the mouse, and with Fleischer's hypothesis of a low-frequency axis parallel to the manubrium. Our observed ratio was significantly lower in three other rats, however, suggesting that the axis was not parallel to the manubrium in those ears.

The frequency responses that we observed at four points on the eardrum are similar in shape to those observed on the manubrium. This suggests that the vibration pattern of the rat eardrum is still simple up to 10 kHz and has not yet broken up into multiple out-of-phase regions.

A more complete analysis of middle-ear function could include phase data and middle-ear output measurements. Since the phase of the frequency response is more difficult to interpret, many authors have performed vibrometry measurements on middle ear structures without reporting the phase data. These studies have been performed in both rats (Doan et al. [1996] and Bigelow et al. [1996, 1998]) and humans (Nakajima et al. [2005] and Gan et al. [2004]). Also, middle-ear output measurements, at the stapes for example, could not be obtained given the experimental setup used.

Conclusion

We have provided a measure of the noise floor and confirmed the linearity of the rat tympanic membrane for sound-pressure levels between 23 and 59 dB SPL. The repeatability of the rat's middle-ear response over time was also investigated. Inter-animal variability at the umbo was presented and compared with other studies. The vibration modes of the tympanic membrane and manubrium were also investigated. By characterizing the vibrations of the manubrium and pars tensa, we have provided information that will contribute to a better understanding of the function of the rat middle ear, and possibly encourage use of the rat in research on the mechanics of hearing.

Acknowledgements

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Captions

Table 1 *Table showing details of the rats used. The right-most column refers to the number of points at which frequency responses were measured in each animal.*

Figure 1 *The rat middle ear. Viewed laterally, with tympanic membrane removed. The rat, and other microtypes, have two axes of rotation, as shown here. (Modified from Zimmer et al., 1994)*

Figure 2 *The osseous skull of the rat, photographed from lateral and ventral angles.*

Figure 3 *Normalized displacements for all seven rats are plotted together. The first measured frequency response is shown for each animal. Inter-ear variability ranges from 20 dB to 40 dB.*

Figure 4 *The variability of our results (grey) is compared with the variability of 5 rats from a previous study (black). Note that velocities are displayed here rather than displacements. (Modified from Bigelow et al., 1996)*

Figure 5 *Frequency responses were measured at the seven points indicated by the stars.*

Figure 6 *Manubrial displacements for a) rat 1, b) rat 2, c) rat 4, and d) rat 6.*

Figure 7 *Manubrial displacements for a) rat 3, b) rat 5, and c) rat 7.*

Figure 8 *Tympanic membrane displacements for a) rat 1, b) rat 2, and c) rat 3.*

Figure 9 *a) Repeat mid-manubrium displacement measurements for rat 4.*
b) Amplitude variation with time at selected frequencies for rat 4.

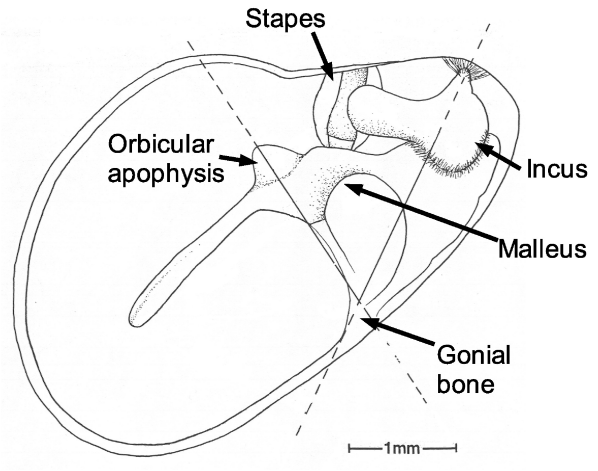
Figure 10 *a) Repeat mid-manubrium displacement measurements for rat 5.*
b) Amplitude variation with time at selected frequencies for rat 5.

Figure 11 *a) Repeat short process displacement measurements for rat 6.*
b) Amplitude variation with time at selected frequencies for rat 6.

Figure 12 *a) Repeat umbo displacement measurements for rat 7.*
b) Amplitude variation with time at selected frequencies for rat 7.

	Sex	Age (months)	Weight (g)	No. points
Rat 1	Male	5	620	7
Rat 2	Female	3	380	7
Rat 3	Female	3	390	7
Rat 4	Male	3	473	3
Rat 5	Male	3	410	3
Rat 6	Male	3	387	3
Rat 7	Female	3	222	3

Table 1



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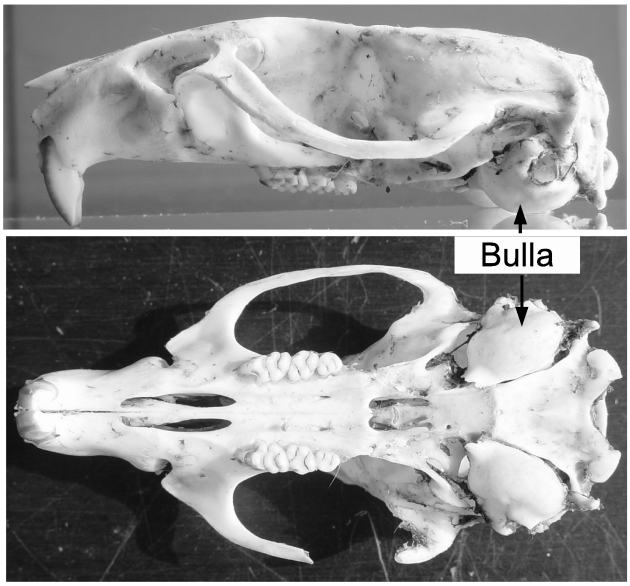

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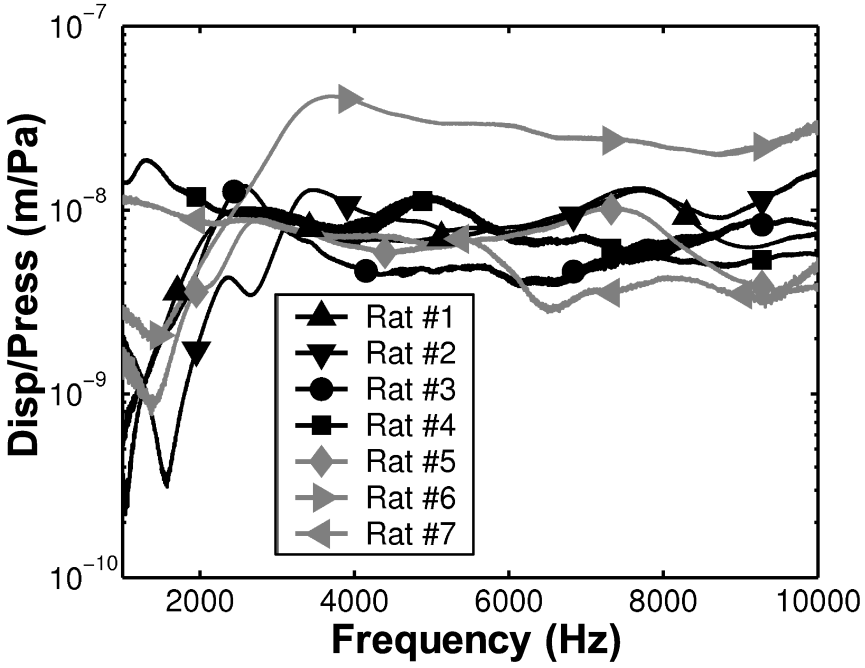

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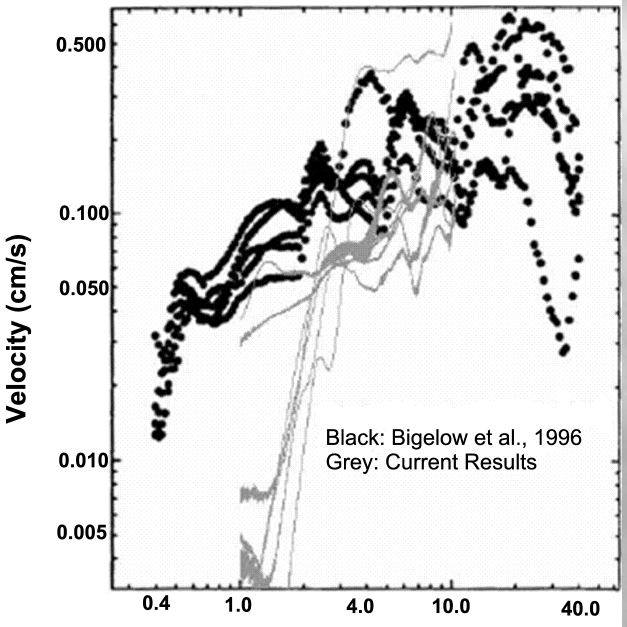

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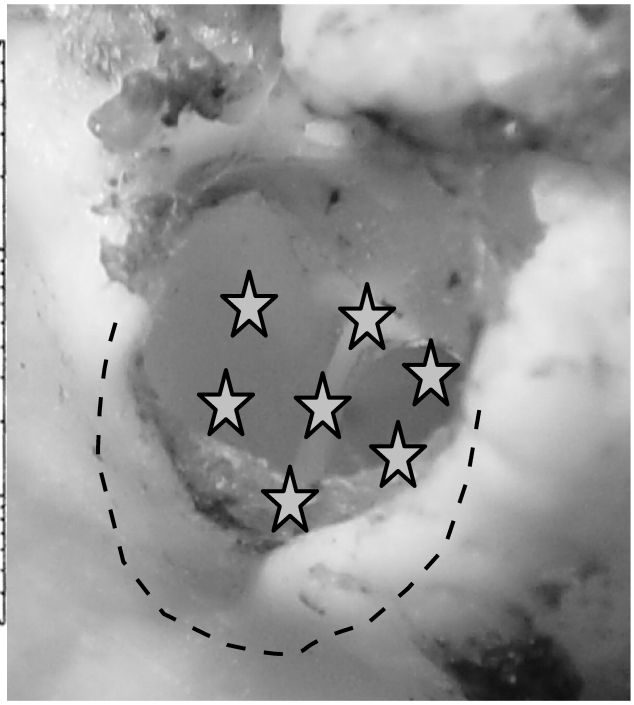

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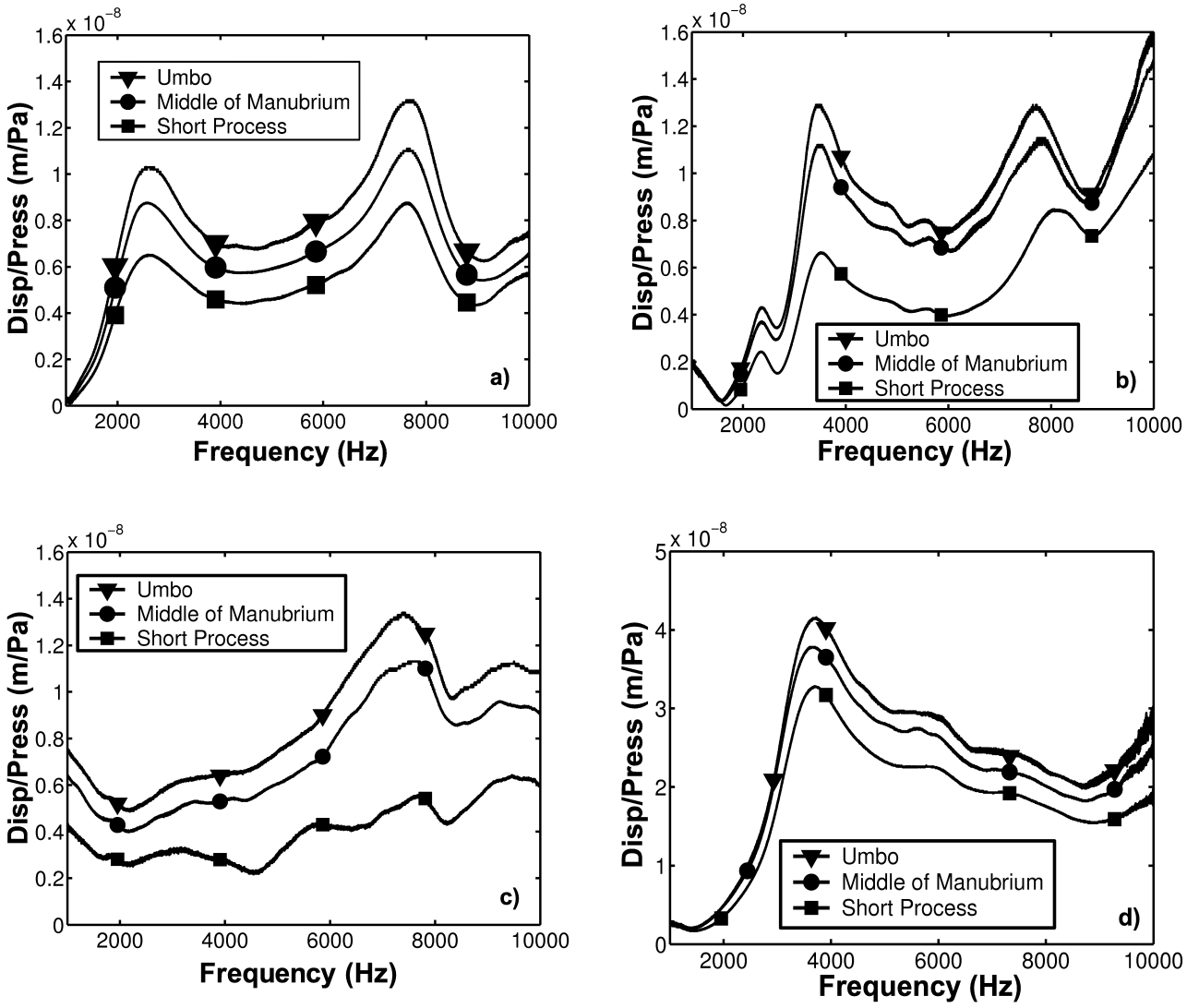
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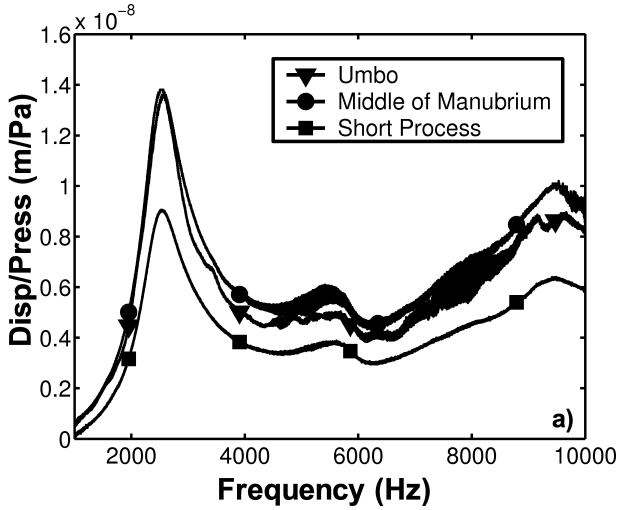
Frequency (Hz)
Umbo
Middle of Manubrium
Short Process
a)
b)
c)
d)

Figure 6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Disp/Press (m/Pa)
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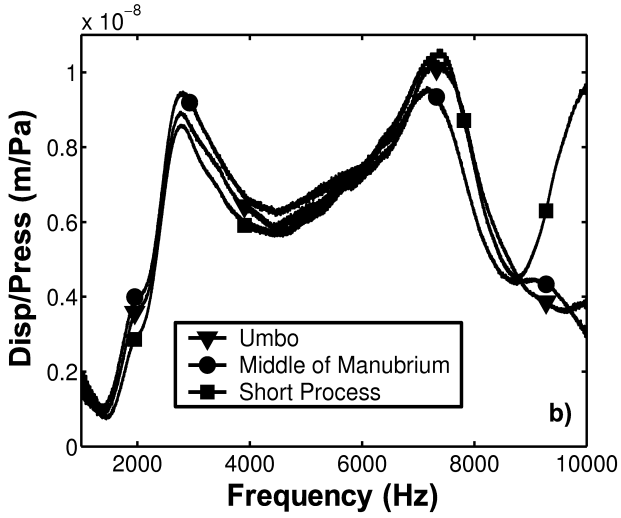
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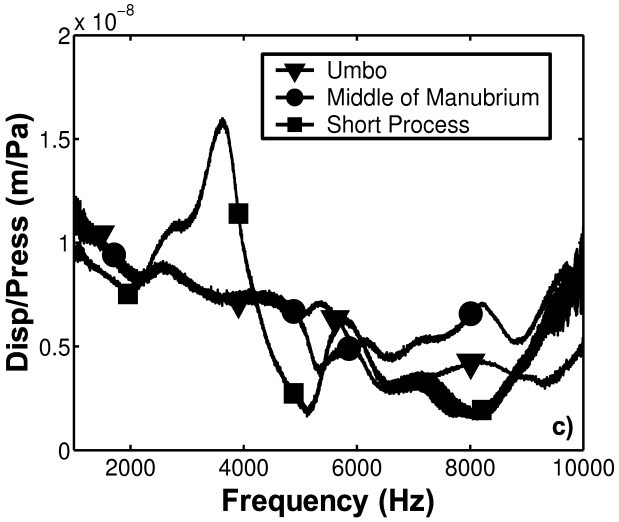
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Disp/Press (m/Pa)
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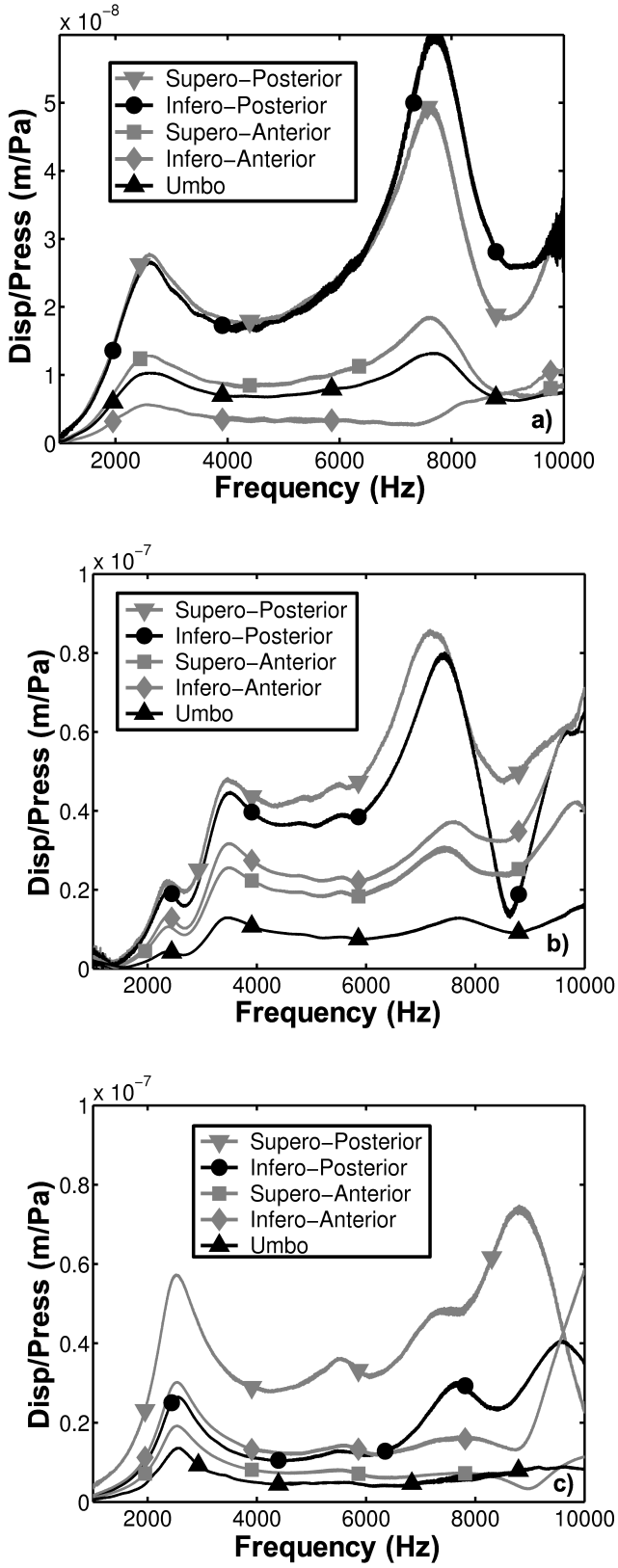
Figure 7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Supero–Posterior
Infero–Posterior
Supero–Anterior
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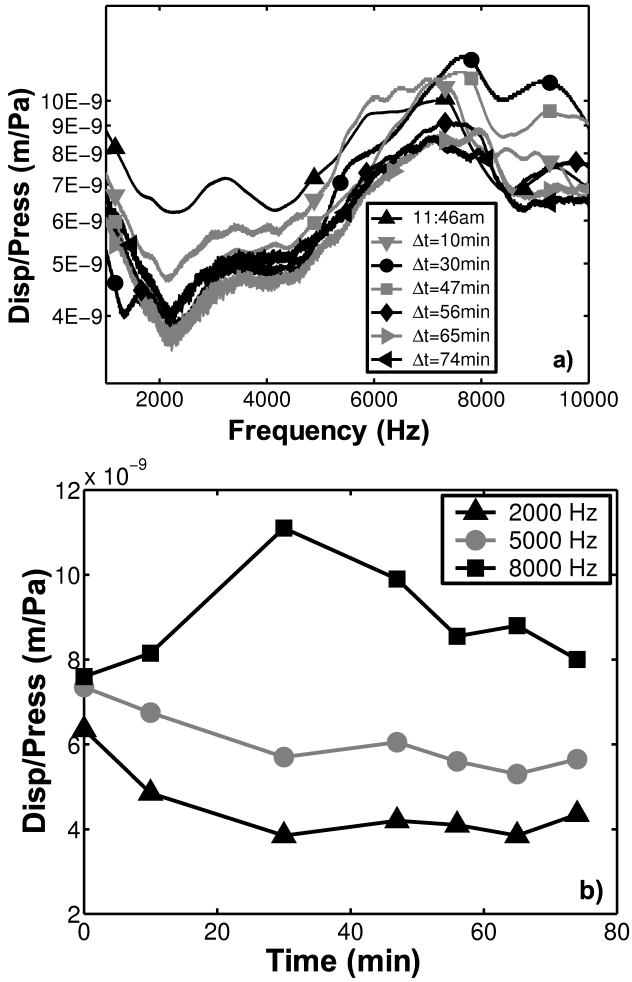
Umbo
Disp/Press (m/Pa)
Frequency (Hz)
a)
x 10^-7
b)
c)

Figure 8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10000
Frequency (Hz)
11:46am
Δt=10min
Δt=30min
Δt=47min
Δt=56min
Δt=65min
Δt=74min
a)
x 10^−9
12
10
8
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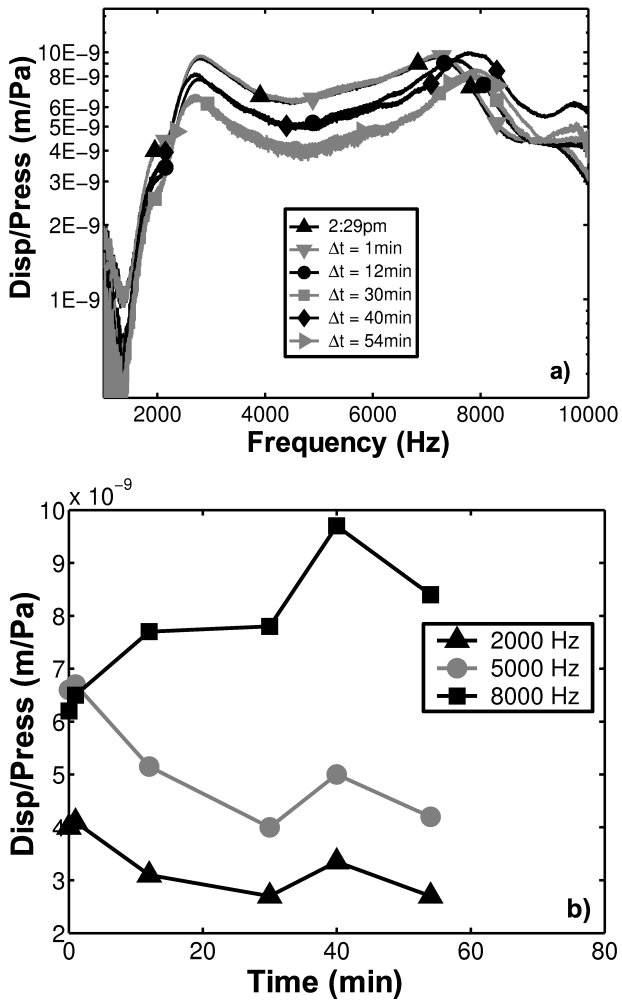
4
2
0
20
40
60
80
Time (min)
2000 Hz
5000 Hz
8000 Hz
b)

Figure 9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Δt = 1min
Δt = 12min
Δt = 30min
Δt = 40min
Δt = 54min
a)
2000
4000
6000
8000
10000
Frequency (Hz)
x 10^−9
Disp/Press (m/Pa)
10
9
8
7
6
5
4
3
2
2000 Hz
5000 Hz
8000 Hz
b)
0
20
40
60
80
Time (min)

Figure 10

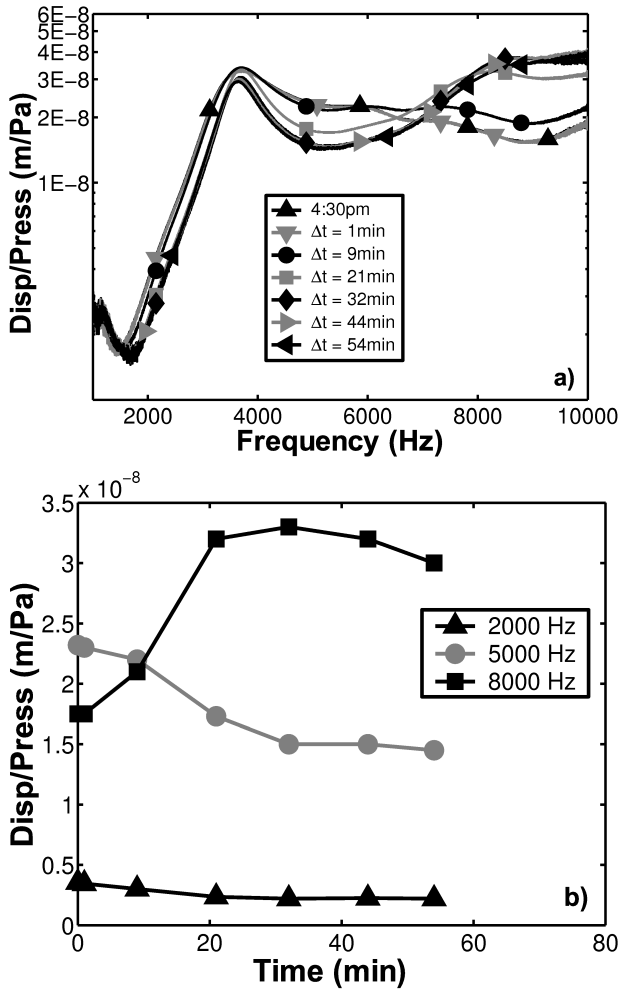
6E−8
5E−8
4E−8
3E−8
2E−8
1E−8
Disp/Press (m/Pa)
4:30pm
Δt = 1min
Δt = 9min
Δt = 21min
Δt = 32min
Δt = 44min
Δt = 54min
a)
2000
4000
6000
8000
10000
Frequency (Hz)
x 10^−8
3.5
3
2.5
2
1.5
1
0.5
0
Disp/Press (m/Pa)
2000 Hz
5000 Hz
8000 Hz
b)
0
20
40
60
80
Time (min)

Figure 11

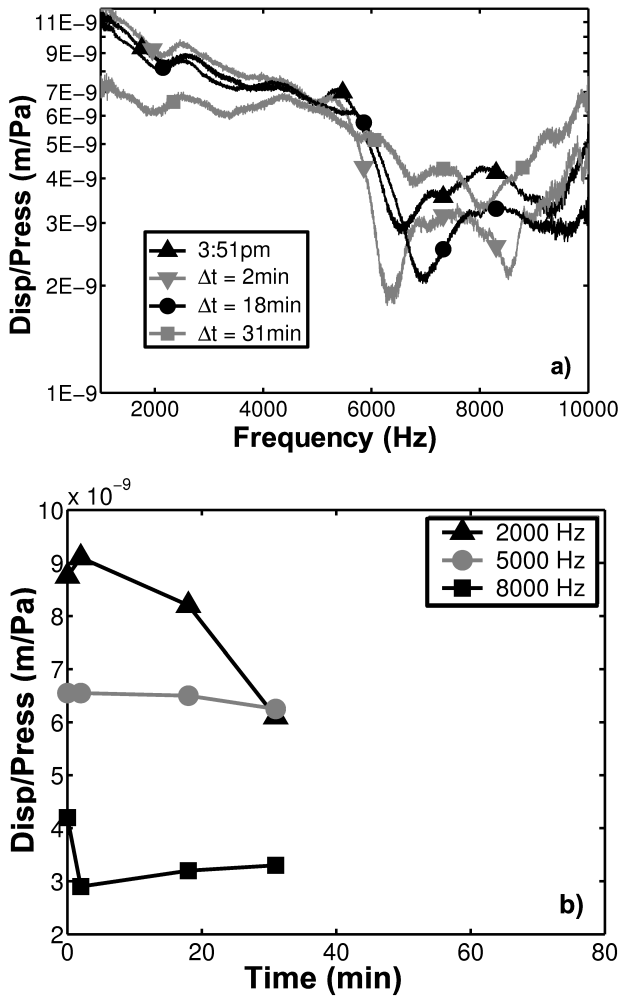
11E−9
9E−9
7E−9
6E−9
5E−9
4E−9
3E−9
2E−9
1E−9
Disp/Press (m/Pa)
3:51pm
Δt = 2min
Δt = 18min
Δt = 31min
a)
2000
4000
6000
8000
10000
Frequency (Hz)
x 10^−9
10
9
8
7
6
5
4
3
2
Disp/Press (m/Pa)
2000 Hz
5000 Hz
8000 Hz
b)
0
20
40
60
80
Time (min)

Figure 12