EXPERIMENTAL STUDY OF THE EFFECTS OF DRYING ON MIDDLE-EAR VIBRATIONS IN THE GERBIL

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INTRODUCTION

The middle ear extends from the eardrum (tympanic membrane or TM) to the oval window, the interface with the inner ear. It includes a chain of three small bones or ossicles (malleus, incus and stapes) suspended in the air-filled middle-ear space. It plays a major role in the hearing process and is the site of many infections and birth defects that contribute to hearing loss. To date, non-invasive diagnostic tools and the quality of middle-ear prostheses are often inadequate. Diagnosis and treatment of hearing loss can be improved with a better understanding of middle-ear mechanics. To this end, many groups have conducted experimental work on mammalian middle ears. Particularly, gerbils have gained great popularity over the past two decades as they are low in cost and have easily approachable middle-ear structures.

Laser Doppler vibrometry (LDV) is a very popular tool in middle-ear research. It permits real-time velocity and displacement measurements of the eardrum and ossicles. LDV measurements are important for a general understanding of middle-ear mechanics and for diagnosing potential middle-ear problems where standard audiological tests fail. LDV has been used in several animal, temporal-bone and live-human studies. Studies by Huber et al. and Rosowski et al. have shown possible diagnostic uses for this technology [1][2]. Akache et al. recently published the first LDV measurements at multiple points on the eardrum [3].

The current study is part of a research effort aiming to characterize the displacements of the gerbil tympanic membrane and middle ear. This paper addresses some important *post mortem* considerations associated with our experimental protocol. After the ear has been dissected to expose the structures of interest, the eardrum and other structures dry out over time, causing changes in material properties. Different strategies to keep the middle ear moist have been discussed by many groups [4][5][6][7][8][9][10]. A few studies have shown that the middle ear can maintain relatively normal behaviour for hours, even days, after death if proper steps are taken.

In a cat study, Khanna and Tondorf suggested that *post mortem* eardrums behave the same as live ones within an hour after death [11]. Rosowski et al. measured impedances in fresh and thawed ears from human cadavers and compared them to *in vivo* data, showing that the middle ear can remain relatively normal for days after death when kept moist [12]. Goode et al. in comparative studies on live and cadaver human ears concluded that no significant differences exist below 6 kHz [13][14]. Voss et al. observed a decrease in magnitude in *post mortem* ears which they attributed to the drying of the TM, the ossicular system or the annular ligament, and found that moistening the middle ear brought the response back almost to its original level [15]. However, few quantitative observations of the effects of drying have been reported.

In this work we designed an experiment to further examine this issue. We collected LDV measurements from a gerbil eardrum at intervals of 5 minutes for a period of 4 hours while periodically remoistening the middle ear. The experiment is designed to provide the first detailed longitudinal tracking of the effect of the drying of middle-ear structures on vibration measurements.

MATERIALS AND METHODS

Specimen Preparation

Measurements were carried out on Mongolian gerbils (*Meriones unguiculatus*) supplied by Charles-River (St-Constant, QC). **Figure 1** shows the anatomy of the gerbil middle-ear and the experimental setup. After the gerbil is sacrificed and decapitated, the lower jaw is completely removed to expose the bulla, the structure enclosing the middle-ear cavity. The external ear is also removed up to the cartilaginous part of the ear canal. Using a surgical drill, parts of the bony ear canal are removed to maximize exposure of the tympanic membrane and to reveal the umbo (the inferior-most point on the manubrium). A large hole is then drilled in the inferior lateral portion of the bulla, revealing the middle-ear cavity. This open-bulla configuration ensures pressure equalization on both sides of the TM. Small wads of absorbent tissue are

placed in the inferior section of the middle-ear cavity (see **Figure 1B**). Rehydration consists of moistening the tissue with a few drops of saline solution, taking care not to have the liquid contact the vibrating structures directly.

Experimental Setup

The gerbil head is affixed with dental cement to a coupler at the opening of the ear canal in an orientation that allows an optimal view of the TM. The coupler is a custom-made aluminium cavity which serves as an acoustically sealed sound chamber. Holes are drilled to allow the insertion of a sound-delivery system (ER-2, Etymōtic Research) and a probe-microphone system (ER-7C, Etymōtic Research) 2 to 3 mm from the TM. A third hole was drilled for a 15-cm PE-10 tube (I.D. = 0.28mm, O.D. = 0.61mm).

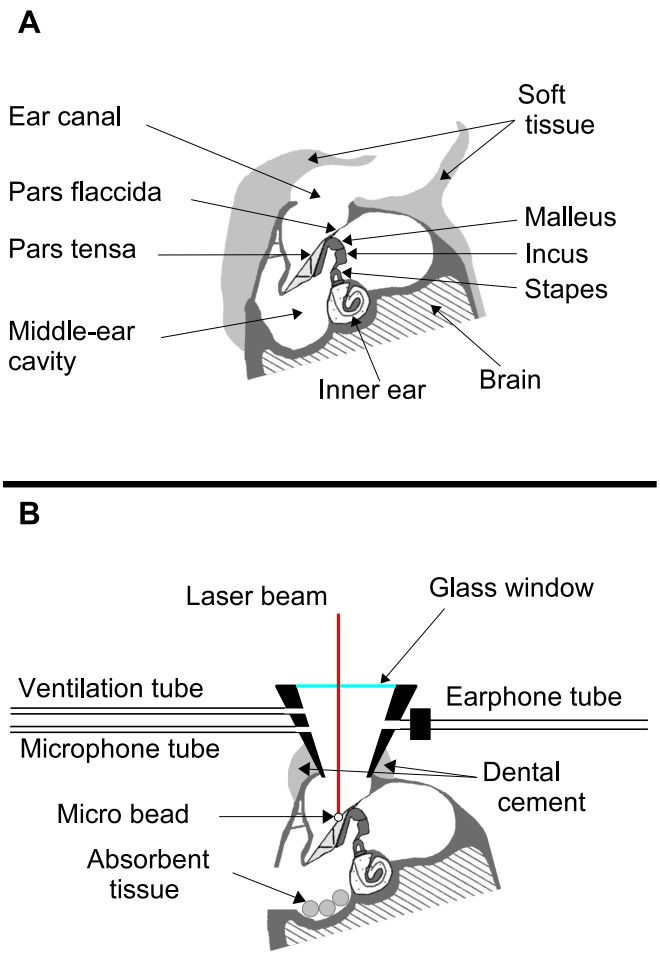

Figure 1: A. Schematic illustration of the gerbil middle ear. Bone is drawn in dark grey and soft tissue in light grey. **B.** Experimental setup: the laser beam is pointed through the glass window and the acoustically sealed cavity and is precisely aimed at a glass bead. (Modified from [16])

This tube acts as a vent to prevent humidity and static pressure from building up inside the coupler while also preventing sound leakage. The top of the cavity is sealed with an antireflection-coated glass window (T47-518, Edmund Optics). The coupler dimensions were designed to prevent acoustical resonances inside the coupler up to 10 kHz (the bandwidth of excitation). The measurements take place inside a double-walled sound-proof room (Génie Audio, St-Laurent, QC) to attenuate interference from outside noise.

Optical Considerations

The experimental preparation is placed under an operating microscope (OPMI 1-H, Zeiss) mechanically coupled to a laser Doppler vibrometer (Polytec HLV-1000). LDV is a type of heterodyne interferometry that measures the interference between a reference beam and a beam reflecting off the surface of interest. It makes use of the Doppler effect to determine the velocity of a vibrating object. Light reflected from a vibrating surface undergoes a shift in frequency proportional to the velocity of vibrations. The vibrometer measures the frequency shift. This sensitive non-contact technique allows reliable measurement of eardrum vibrations on the nanometer level, without loading the middle ear. The HLV-1000 was specifically developed to measure vibrations in the middle ear and hearing devices. The system consists of a laser Doppler vibrometer unit, a sound-delivery source, and a data acquisition and analysis system connected to an Intel®-based computer.

The operation of the vibrometer requires a sufficient amount of light reflecting off the surface of the TM. However, the high anisotropy coefficients of biological tissues (between 0.9 and 0.99) cause much light to be lost to forward scattering [17]. The slope of the surface of the TM further reduces the amount of back-scattered light. To compensate, we place glass micro beads (diameter 90-150 μm, Sigma) at the points of measurement (see **Figure 2**) to increase the amount of back-scattered light and improve the signal-to-noise ratio in the measured signal. These micro beads adhere to the TM by simple capillary force. Their use has been validated by Decraemer et al. [18].

Measurements

Measurements are taken starting about 2 hours after the animal is sacrificed. This is the time it takes to prepare and mount the specimen. We use a 128-ms sinusoidal-sweep excitation signal over the frequency range of 150 Hz to 10 kHz. The microphone monitors the sound pressure level (SPL) within the coupler, while the vibrometer signal contains vibration velocity data. Displacement transfer functions are obtained by normalizing the vibrometer signal against the SPL and

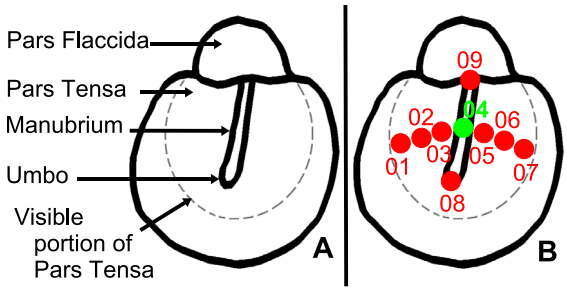

Figure 2: A. Schematic representation of the gerbil tympanic membrane. Outlined in grey is the area visible under the microscope through the enlarged opening of the ear canal. **B.** Location of measurements.

dividing by the frequency. Measurements were carried out on 4 gerbils. Averages of 100 measurements were taken at the points shown in **Figure 2** in the following sequence:

- Step 1: Two sets of data (A & B) were collected sequentially at all 9 points on the TM and manubrium. The absorbent tissue was moistened about 25 minutes before this step.
- Step 2: Single-point measurements were collected midway along the manubrium (point 4) at 5-minute intervals. The absorbent tissue was remoistened twice during this step.
- Step 3: Two sets of data (C & D) were collected sequentially at all 9 points again.
- Step 4: One final measurement (E) was collected at point 4.

RESULTS

We present here only the repeated measurements taken at point 4 on one gerbil (10 weeks old, male, 72 g). **Figure 3** shows normalized displacements from 150 Hz to 10 kHz. The frequency response features a relatively flat response at low frequencies, a heightened sensitivity around 6500 Hz and a higher peak around 9500 Hz. The three curves in the figure correspond respectively to measurements taken at the beginning of the experiment (t = 0, that is, during the series of measurements A), then just before (t = 99 minutes) and just after (t = 104 minutes) remoistening the absorbent tissue the first time.

The second curve shows a decrease in magnitude and a shift of the peaks to higher frequencies. This frequency shift is more observable at high frequencies. The third curve, after rehydration, shows the response to be partially restored to the original level. The spectrogram in **Figure 4** shows the progression of the frequency responses over 4 hours. Frequencies below 4 kHz are omitted in order to better reveal the frequency shifts of the peaks. **Table 1** provides a quantitative summary of these changes with respect to the original measurement. A considerable magnitude drop can be observed at the low frequency peak with a negligible frequency shift. At the high frequency peak, the magnitude drop is proportionally smaller, with a significant frequency shift.

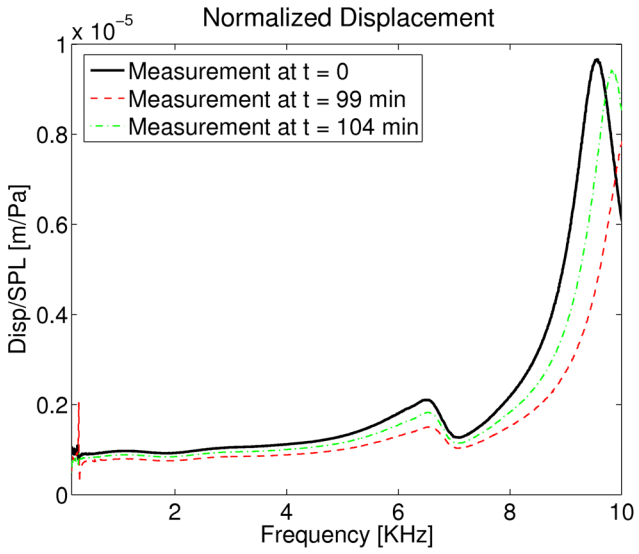

Figure 3: Three curves of normalized displacement of gerbil mid-manubrium vibrations at times t = 0, 99 & 104 minutes. Remoistening of absorbent tissue with saline solution occurred at t = 100 minutes.

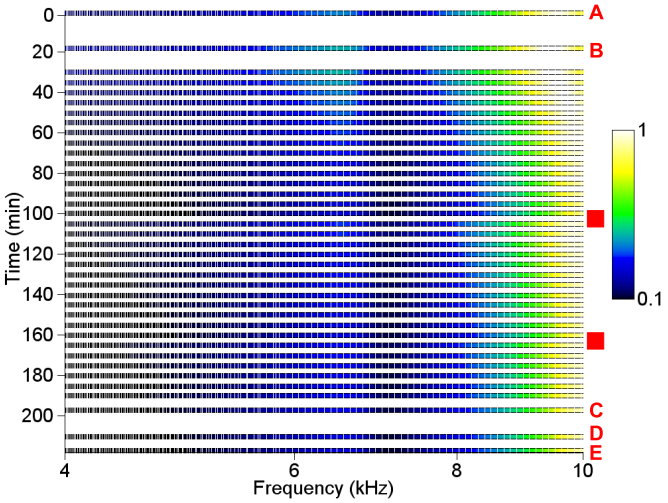

Figure 4: Spectrogram showing the distribution of amplitudes as a function of frequency (on a log scale) and time. The colour scale encodes amplitudes normalized to 10 µm. On the right, letters indicate measurement samples taken during steps 1, 3 and 4 (see text). Squares (■) indicate the times of remoistening the absorbent tissue (t = 100 & 160).

Table 1: Summary of magnitude drops and frequency shifts at the low and high frequency peaks on the curve.

	Low frequency peak		High frequency peak	
	Magnitude drop	**Frequency shift**	**Magnitude drop**	**Frequency shift**
Before first rehydration	28%	< 50 Hz	15%	600 Hz
After first rehydration	13%	< 30 Hz	2.4%	266 Hz
Before second rehydration	26%	50 Hz	13%	684 Hz
After second rehydration	17%	~ 0 Hz	6.4%	508 Hz

CONCLUSION

This paper presents an assessment of *post mortem* middle-ear behaviour over a period of 4 hours, and of the effects of drying and rehydration. Our results quantitatively support what was suggested by Voss et al.: by remoistening the middle ear, *post mortem* effects of drying of the middle ear can be partially reversed [15].

There may be *post mortem* effects other than drying which affect the material properties and contribute to the changes observed. Further efforts are required to investigate this possibility, and also the changes that occur during the time between sacrificing the animal and the first measurements.

Our research makes use of LDV measurements to validate finite-element models. An important aspect of this work is to compare simulated vibration patterns with the multiple LDV measurements at a large number of locations which are required to characterize the 3-D vibration patterns of the TM and ossicles. Such multiple measurements are time-consuming, however, which leads to drying effects, making comparisons with the model potentially misleading. Systematic observations of the time course of the effects of drying, as presented here, could be used to correct for the time effects and thus facilitate the comparison with simulation results.

Conversely, it would also be possible to use a finite-element model in order to understand certain features of the observed *post mortem* effects. By adjusting the model parameters to simulate drying effects, we can investigate questions such as why the frequency of the higher-frequency peak is more sensitive to drying than is that of the lower-frequency peak.

ACKNOWLEDGEMENTS

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