AN EXPERIMENTAL STUDY OF MIDDLE-EAR VIBRATIONS IN RATS

Fadi Akache,[1] Sam J. Daniel,[2] W. Robert J. Funnell[1, 2]

[1] Department of BioMedical Engineering, McGill University
[2] Department of Otolaryngology, McGill University
Montréal, QC, Canada H3A 2B4

ABSTRACT

Hearing impairment is one of the most common disabilities. Animal models have been shown to be valuable tools in auditory research, including studies of middle-ear mechanics. Rats are potentially very useful for this purpose. They are low in cost, they are genetically similar to humans, and the middle-ear structures are easily approachable. The goal of the present study is to characterize the frequency response of the rat tympanic membrane. A laser Doppler vibrometer was used to measure the vibrations of the tympanic membrane. Measurements were done on two female Sprague Dawley rats. The tympanic membrane was stimulated with 45 pure-tone sine waves between 250 and 8000 Hz in Rat #1 and with a slowly sweeping signal from 200 – 10 000 Hz in Rat #2. Results at the umbo compare well with previous studies. The first measurements of the frequency response of the rat tympanic membrane at points other than the umbo are also presented.

INTRODUCTION

Hearing impairment affects up to 30% of the population. It is also one of the most common birth defects, affecting 3 in 1 000 babies. Hearing loss is the third leading chronic disability after arthritis and hyperextension. The middle ear plays a major role in the hearing process. There is still much about the ear that remains to be understood, and with a better understanding of the mechanics of the middle ear, we will be able to improve the diagnosis and treatment of hearing disorders.

The middle-ear consists of the eardrum, or tympanic membrane (TM), and three tiny bones, or ossicles, known as the malleus, the incus and the stapes. The upper portion of the TM is known as the pars flaccida (PF) and the lower part as the pars tensa (PT). The TM is roughly conical, pointing inward, and the deepest point is known as the umbo. The manubrium of the malleus is attached to the medial side of the TM, and extends down as far as the umbo. The tympanic membrane converts sound pressure at its outer surface into movement of the malleus and, consequently, the entire ossicular chain.

It has been suggested by Hellström et al. (1982) that the rat is of value in otological research because the middle-ear structures are easily approachable. Rats are also 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 with the recent addition of rats 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.

Few measurements have been done in the past to study 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. Moreover, these researchers did not have access to today's more advanced measurement techniques. More recent studies by Doan et al. (1996) and Bigelow et al. (1996, 1998) did provide the frequency responses and used technology similar to the one presented in this paper, but they measured the frequency responses only at the umbo. Although umbo measurements can reveal, in part, how the middle ear functions, they do not describe tympanic-membrane vibration patterns. To fully understand the vibrations of the TM, one needs to measure displacements at points other than the umbo.

The previous measurements in the rat were all done with pure-tone sine waves as the stimulus. The time-consuming nature of this stimulation method does not allow for a high frequency resolution in reasonable amounts of time. The previous studies provided no more than 130 stimuli over a 40-kHz range. By applying a slowly sweeping sine wave, it is possible to obtain many thousands of readings, dramatically improving the frequency resolution.

The goal of the present study is to characterize the frequency response of the rat tympanic membrane. Results were obtained using both pure-tone and slow-sweeping sinusoidal stimulation. Measurements were taken both at the umbo and at points on the tympanic membrane.

MATERIALS AND METHODS

Specimen Preparation

The measurements were carried out on two Sprague Dawley rats supplied by Charles-River (St-Constant, QC). Measurements were taken within three hours from the time 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 exposure 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, a small hole was drilled into the bulla, equalizing the pressures on the two sides of the TM. Figures 1 and 2 illustrate the location of the tympanic bulla in the rat's skull and the anatomy of the tympanic membrane, respectively.

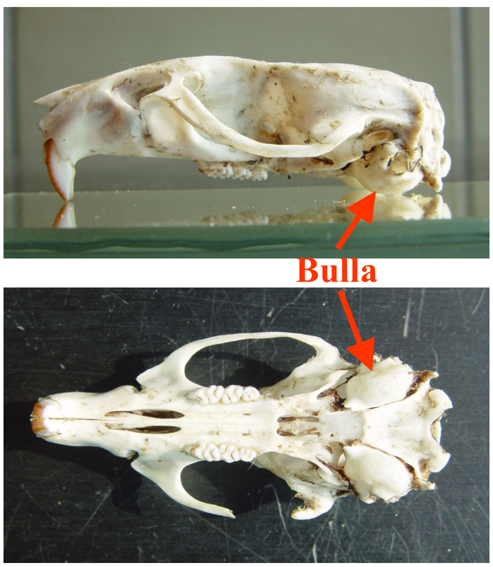

Figure 1 The osseous skull of the rat, photographed from lateral and ventral angles

Figure 2 Photograph of right tympanic bulla and tympanic membrane viewed from the lateral side. 1 = Right tympanic bulla, 2 = pars flacida, 3 = pars tensa. (Hellstrom et al., 1982)

In these experiments, the area of interest was kept moist with saline solution. Several studies have shown that the middle ear can remain relatively normal for hours, or even days, after death. Measurements reported by Rosowski (1990) and the results of Khanna and Tonndorf (1972) in the cat also suggest that the middle ears of live and post-mortem subjects behave similarly.

	Rat #1	Rat #2
Sex	Female	Female
Weight	350g	346g
Ear	Right	Right
Stimulus	45 Pure-tones	Sweep

Table 1

Acoustical System

A 0.5-cc cylindrical sound chamber was placed around the TM and the surrounding region was sealed with Plasticine™ to prevent drying and sound loss. An ER-2 Tubephone™ (Etymōtic Research) was used as the sound delivery system. In Rat #1, 45 pure-tone sine waves were administered at 80 dB SPL; Rat #2 was subjected to a slowly sweeping sine signal (200 – 10 000 Hz) at 40 dB SPL. The sound pressure level was monitored with an ER-7C (Etymōtic Research) probe-microphone system placed 2-3 mm from the TM. Both the sound-delivery system and the probe-microphone system were inserted into the sound chamber, which was sealed with a glass cover slip. The setup is shown in figure 3. Measurements were done within a double-walled audiometric sound room (Génie Audio, St-Laurent, QC) to attenuate any outside noise that might compromise the measured frequency responses.

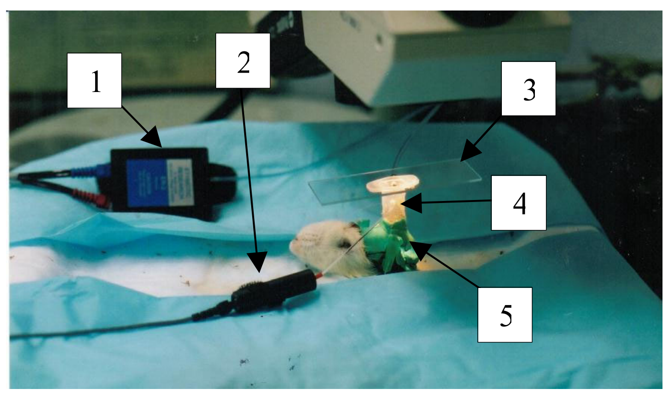

Figure 3 Photograph of experimental setup. 1 = ER-2 Tubephone™, 2 = ER-7C probe microphone system, 3 = glass cover slip, 4 = sound chamber, 5 = Plasticine™

Optical System

Displacements were measured using a laser Doppler system (Polytec HLV 1000) coupled to an operating microscope (Zeiss, OPMI-1). Laser Doppler vibrometry provides a reliable method of measuring tympanic-membrane vibration: it is a sensitive non-contacting optical technique that does not load the middle ear and it is capable of measuring displacements on the nanometer level (Goode et al., 1993). Polytec developed the HLV-1000 Hearing Laser Vibrometer specifically for measuring frequency responses in the middle ear and in hearing devices. The system consists of a compact laser Doppler vibrometer (LDV) mounted to an operating microscope; an excitation source; and a complete data acquisition and analysis system. Laser Doppler vibrometry has been used by many groups for middle-ear research.

As the name suggests, a laser Doppler vibrometer makes use of the Doppler effect to measure the vibrations of the object of interest. The Doppler effect is the change in the observed frequency of a wave due to relative motion between the source and the observer. Similar to how a car moving quickly with its horn blowing will result in a changing of the note's observed frequency, light scattered back from the surface of the vibrating tympanic membrane is shifted in frequency by an amount proportional to the velocity of the surface. The LDV measures this shift and produces a velocity signal to be analyzed. Low-frequency noise is a problem when using this technique due to the inherently low velocities at low vibration frequencies.

Laser vibrometry requires a sufficient amount of backscattered 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 a glass micro bead (90 – 150 μm in diameter, Sigma) on the tympanic membrane. The micro bead was attached by simple capillary force. Given the negligible mass of this bead, we were able to increase signal-to-noise ratio without affecting the frequency response.

RESULTS

The measured frequency responses are presented in figures 4 and 5. The responses were recorded during the pure-tone and slow-sweep stimulations with the laser Doppler vibrometer. Both rats show heightened sensitivity in the lower frequency range at approximately 3 000 Hz and a peak in the higher frequency range, at approximately 7 500 Hz for Rat #1 and 7 000 Hz for Rat #2 (figure 4).

Displacements at three different points on the TM were measured for Rat #2. Measurements were taken at the umbo, at one point on the center of the pars flaccida (PF) and at an antero-superior point on the pars tensa (PT) (figure 5). While both the umbo and PT show similar peaks near 3 000 Hz, there is also a large, sharp peak on the PT, at approximately 7 300 Hz, which is not seen at the umbo. The PF frequency response shows a peak at 2 800 Hz which is not seen on either the PT or the umbo.

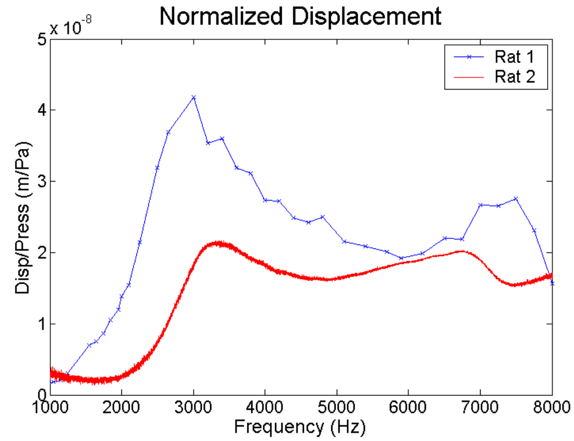

Figure 4 Normalized displacements at the umbo for Rat #1 and Rat #2

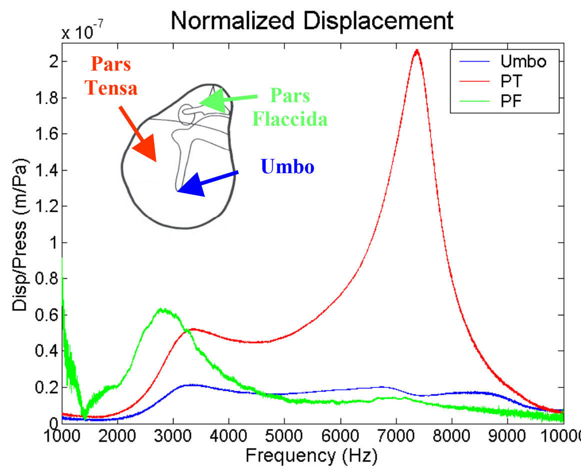

Figure 5 Normalized displacements for Rat #2 at the umbo, pars tensa, and pars flaccida.

DISCUSSION & CONCLUSIONS

Beccari and Molinengo (1958) and Ishii et al. (1964) found that the frequencies of maximum sensitivity of the ear of rats of the Wister strain were about 1 500, 7 000-7 500, and 15 000 Hz. More recent studies on Long-Evans rats by Doan et al. (1996) showed that three peaks, at about 2 300, 6 300, and 20 000 Hz, characterized the rat's umbo velocity responses. Bigelow et al. in 1996 reported the presence of three peaks in the velocity response of Long-Evans rats at 2 500, 5 500, and 15 000 Hz, and then, in 1998, reported peaks at 1 500, 5 000, and 14 000 Hz. Our results compare reasonably with these findings, showing peaks at about 3 000 and 7 000-7 500 Hz. Although the differences could be attributed to inter-subject or inter-strain variability, further study is needed to confirm this.

No studies have previously been done to determine the frequency response of the tympanic membrane of rats at points other than the umbo. Figure 5 shows the displacements at the umbo, pars flaccida (PF) and pars tensa (PT) for Rat #2. These results show that the PT displaces more than the umbo. The common peak at approximately 3 kHz may reflect an ossicular resonance at that frequency. There is a large peak on the PT, at approximately 7 300 Hz, which is not reflected in the umbo frequency response. One would expect that measurements at different points on the PT would show peaks at different frequencies.

The PF displacements seem to be quite different from those at the umbo and PT, consistent with an uncoupling as a result of the different properties and orientations of the PT and PF. Further measurements will be needed to validate these findings.

An important factor in frequency-response measurements of the tympanic membrane is the choice of stimulus. An ideal stimulus would result in high signal-to-noise ratios (SNR) with short measurement times. Although the steady-state sinusoidal pure-tones provide the best SNR, obtaining results is a very time-consuming process, especially if a high frequency resolution is desired. The slowly sweeping signal gives a lower signal-to-noise ratio when compared with the pure tones, but provides higher resolution in less time.

As predicted, the frequency response obtained with a slow-sweeping signal (Rat #2) is noisier than that with the pure-tone stimulation (Rat #1), especially at the lower frequencies. Doan et al. (1996) demonstrated that measurements at the lower frequencies would be clouded by noise at intensities below 70 dB SPL. Since our current setup only allowed us to input a 40-dB signal in the slow-sweeping experiment with Rat #2, as opposed to 80 dB SPL with the pure tones, the response at the lower frequencies was noisy.

The differences between the two responses can be attributed to individual variations in TMs and middle ears. Considerable variations were found in Doan et al.'s (1996) results as well. Bigelow et al. (1996, 1998) did not examine inter-subject variability but their results in 1996 were considerably different from those in 1998. Human studies have also demonstrated large inter-subject variations in frequency responses. Using a larger number of subjects should demonstrate that the variability was not a result of the different stimuli.

Our experience has confirmed that the middle-ear structures are, in fact, easily approachable in the rat. Since the rat is also considerably less expensive than most other mammalian experimental animals, we believe that it could be of great value in ear research. It will be necessary, however, to investigate the feasibility of experiments involving modification of the middle-ear structures, given the smaller size of the rat ear.

REFERENCES

Albiin N., Hellström S., Salén B., Stenfors L.E., Söderberg O. (1983): *The anatomy of the Eustachian tube in the rat: a macro- and microscopial study*. Anat Rec 207: 513-21

Beccari E., Molinengo L. (1958): *Osservazioni sulla sensibilita' uditiva del ratto alle diverse frequenze di stimolazione e sulla capacita' discriminitiva della intensita'*. Soc. Ital. Biol. Sperim. Boll. 34: 368-71

Bigelow D.C., David K., Saunders J.C. (1998): *Effect of healed tympanic membrane perforations on umbo velocity in the rat.* Ann Otol Rhinol Laryngol 107: 928-34

Bigelow D.C., Swanson P.B., Saunders J.C. (1996): *The effect of tympanic membrane perforation size on umbo velocity in the rat.* Laryngoscope 106(1): 71-76

Doan D.E., Igic P.G., Saunders J. (1996): *Middle-ear development VII: Umbo velocity in the neonatal rat*. J. Acoust. Soc. Am. 99 (3), 1566-72

Goode R.L., Ball G., Nishihara S. (1993): *Measurement of umbo vibration in human subjects – method and possible clinical applications*. Am J Otol 14(3):247-51

Gyo K., Goode R.L., Miller C. (1986): *Effect of middle ear modification on umbo vibration.* Arch Otolaryngol Head Neck Surg 112: 1262-8

Hellström S., Salén B., Stenfors L.E. (1982): *Anatomy of the Rat Middle Ear. A study under the dissection microsope.* Acta anat 346-52

Huber A.M., Schwab C., Linder T., Stoeckli S.J., Ferrazzini M., Dillier N., Fisch U. (2001): *Evaluation of Eardrum Laser Doppler Interferometry as a diagnostic tool.* Laryngoscope 111: 501-07

Ishii H., Kimura K., Harada O. (1964): *Stress and auditory characteristics of the rat*. Ann. Otol. Rhinol. Laryngol. 73: 948-56

Khanna and Decraemer (1997): *Vibration Modes and the Middle Ear Function. pp. 21-26* in Hüttenbrink K.B. (ed): Middle ear mechanics in Research and Otosurgery. Proceedings of the International Workshop on Middle Ear Mechanics, Dresden, Germany, 19-22 Sept, 1996. Dresden, University of Technology

Khanna S.M., Tonndorf J. (1972): *Tympanic membrane vibrations in cats studied by time-averaged holography*. J Acoust Soc Am 51: 1904-20

Rat Genome Sequencing Consortium (2004): *Genome sequence of the Brown Norway rat yields insights into mammalian evolution*. Nature 428: 493-521

Rosowski J.J. (1990): *Cadaver Middle Ears as Models for Living Ears: Comparisons of Middle Ear Input Immittance*. Ann Otol Rhinol Laryngol 403-12

Rosowski J.J., Brinsko K.M., Tempel B.I., Kujawa S.G. (2003): *The aging of the Middle Ear in 129S6/SvEvTac and CBA/CaJ Mice: Measurements of Umbo Velocity, Hearing Function, and the Incidence of Pathology*. JARO 04: 371-83

Sesterhen G., Breuninger G. (1978): *On the influence of the middle ear muscles upon changes in sound transmission*. Arch Otolaryngol 221: 47-60.

Vogel U., Zahnert T., Hofmann G., Offergeld C., Hüttenbrink K.B. (1997): *Laser Vibrometry of the Middle Ear: Opportunities and Limitations. pp. 128-33* in Hüttenbrink K.B. (ed): Middle ear mechanics in Research and Otosurgery. Proceedings of the International Workshop on Middle Ear Mechanics, Dresden, Germany, 19-22 Sept, 1996. Dresden, University of Technology