Reverse Engineering the Cetacean Ear to Extract Audiograms

Aleks Zosuls, Seth O. Newburg, Darlene R. Ketten, and David C. Mountain

1 Introduction

The cochlear frequency-place map is believed to be an important determinant of the frequencies that a species can hear as well as the bandwidth of cochlear filters. Both features impact an animal's ability to detect biologically significant sounds in noise. The cochlear frequency-place map is created in part by a stiffness gradient in the basilar membrane (BM) in which stiff regions respond best to high frequencies and more compliant regions respond best to low frequencies.

The goal of this research is to build cochlear models that predict audiograms of species for which it is impractical to obtain an audiogram through behavioral testing (e.g., large marine mammals). In this study, we measured BM stiffness in *Tursiops truncatus, Meriones unguiculatus, Phocoena phocoena,* and *Delphinus delphis,* all species with known audiograms. The results will be used to calibrate cochlear models for estimating the audiograms of species that cannot be measured behaviorally.

2 Methods

A custom piezoelectric force probe was constructed based on Olson and Mountain (1991) and Naidu and Mountain (1998). The probe consists of two displacement transducers and a force sensor in series, terminating at a sharp tip placed in contact with the underside of the BM. The first displacement transducer was mounted to a micromanipulator and was used to apply static displacements to the probe, displacing the probe tip toward the membrane in 1-mm steps. The second displacement transducer applied a 50-nm peak-to-peak 80-Hz sinusoidal signal to the probe tip.

A. Zosuls (✉) • D.C. Mountain
Boston University, Boston, MA 02215, USA
e-mail: azosuls@bu.edu; dcm@bu.edu

S.O. Newburg
Massachusetts Institute of Technology, Cambridge, MA 02142, USA
e-mail: setho@mit.edu

D.R. Ketten
Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
e-mail: dketten@whoi.edu

A.N. Popper and A. Hawkins (eds.), *The Effects of Noise on Aquatic Life,*
Advances in Experimental Medicine and Biology 730, DOI *10.1007/978-1-4419-7311-5_13,*

The force sensor was a piezoelectric bimorph with the glass probe tip bonded to its center. As the second displacement sensor applied the sinusoidal stimulus, the force sensor measured the return force of the membrane. A computer with Tucker-Davis Technologies and National Instruments data-acquisition hardware running custom MATLAB scripts was used to control the experiments.

Inner ear preparations varied by species. In *Meriones,* the animals were deeply anesthetized, then decapitated according to Institutional Animal Care and Use Committee-approved protocols. The bulla was removed and placed in oxygenated L-15 culture medium (Sigma-Aldrich). The scala tympani was opened, exposing the underside of the BM, and mounted on a holder with cyanoacrylate glue (Great Planes). The force probe was positioned orthogonal to the BM using a surgical microscope. A radial profile of positions was obtained by scanning from the spiral lamina to the spiral ligament. Longitudinal location was recorded by digital images. In *Meriones,* only one longitudinal location was taken per preparation to ensure the most physiologically relevant data.

In *Tursiops, Phocoena,* and *Delpinius,* a different approach was required because the ears were harvested postmortem. Legal restrictions prevent euthanasia perfusion for research; therefore, fresh samples were obtained opportunistically. Fixatives can also change mechanical tissue properties. After an animal was pronounced dead naturally or euthanized for medical reasons, its ears were extracted at the site of stranding or at the Marine Mammal Facility, Woods Hole Oceanographic Institution, Woods Hole, MA, scanned in a CT unit, and transported immediately to Boston University, Boston, MA, for measurement. In many cases, the experiments were performed 8-24 h postmortem.

The bullar complex of marine mammals is composed of dense, fully ossified bone, second only to teeth in density and hardness. A Dremel Moto tool and a dental drill equipped with carbide burrs were used to open the scala tympani to expose the underside of the BM. During this process, the ear was bathed in normal saline solution to cool and maintain moisture. Bone dust was removed by vacuum to prevent contamination of the BM. The periotic bone was ground very near to the canals. The remaining bone was carefully chipped with a scalpel to minimize spiral lamina, spiral ligament, and BM damage. The ear was then mounted on a large ear bar with cyanoacrylate glue. The bar was positioned under the probe, and a radial profile was collected. Longitudinal location was documented with photographs. The ear was removed from the probe system, and a new longitudinal access location was opened in the bone. During machining, existing holes were sealed with bone wax to prevent contamination with bone dust. The process was repeated for multiple locations base to apex until the preparation deteriorated or the cochlea collapsed.

3 Results

In all ears measured, the stiffness values decreased from base to apex (Fig. 1). Higher frequency species had the highest basal tum stiffness (Fig. 1). These results are consistent with other measurements made on BM stiffness.

Probe noise floor limited the ability to make reliable measurements in the very low stiffness apical regions. New strategies are being developed to measure these regions in a timely matter. They currently require many averages and finer probe advancing steps, which is problematic with the rapid deterioration of the tissue.

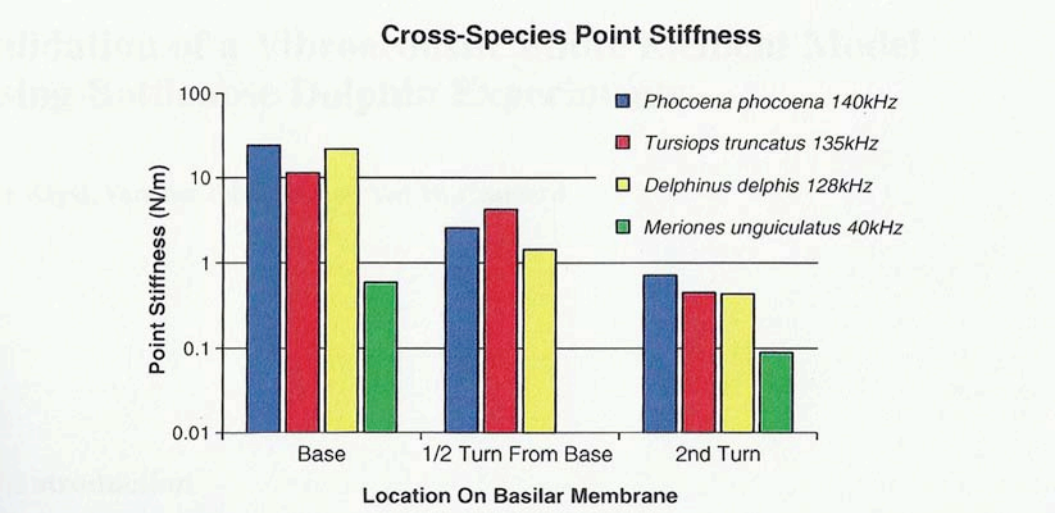

Fig. 1 Point stiffness for multiple species at three longitudinal locations along the length of the basilar membrane. Radial data from each longitudinal position were averaged. Frequencies refer to high-frequency cutoffs in measured audiograms of *Phocoena* (Kastelein et al. 2002), *Delphinus* (Popov and Klishin (1998), *Tursiops* (Ljungblad et al. 1982), and *Meriones* (Ryan 1976)

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