Aquatic Mammals 2007, *33*(1), 93-99, DOI 10.1578/AM.33.1.2007.93

A Portable System for the Evaluation of the Auditory Capabilities of Marine Mammals

Kristen A. Taylor,[1,2] Paul E. Nachtigall,[1] T. Aran Mooney,[1,2] Alexander Ya. Supin,[3] and Michelle M. L. Yuen[4]

[1]*Marine Mammal Research Program, Hawaii Institute of Marine Biology, University of Hawaii, P.O. Box 1106, Kailua, HI 96734, USA; E-mail: kristent@hawaii.edu*
[2]*Department of Zoology, University of Hawaii, 2538 The Mall, Edmonson 152, Honolulu, HI 96822, USA*
[3]*Institute of Ecology and Evolution, Russian Academy of Sciences, 33 Leninsky Prospect, 119071 Moscow, Russia*
[4]*National Marine Fisheries Service, Pacific Islands Regional Office, 300 Ala Moana Boulevard, Honolulu, HI 96850, USA*

Abstract

We have created a portable system that is capable of measuring the hearing thresholds of marine mammals. It was designed for the purpose of testing the auditory capabilities for a wide range of marine mammal individuals and species. This system consists of multiple individual components, independently purchased or assembled. The major component of the system is a standard laptop computer with custom software that is able to both generate outgoing signals and acquire the corresponding brain potential measurements in response to those outgoing signals. The system has been, and still is, in an ongoing state of improvement and optimization with the goal of having a final system that could be used in nearly all field conditions.

Key Words: auditory evoked potential (AEP), marine mammal, threshold

Introduction

Auditory evoked potentials (AEPs) are fluctuations in the normal electroencephalogram (EEG) when neuronal units in the brain respond to an acoustic stimulus. AEP techniques were developed for human infants as a quick and quantitative assessment of baseline hearing capabilities (Sohmer & Feinmesser, 1967; Jewett et al., 1970, 1971; Hall, 1992). Due to the limited number of marine mammals in captivity and the time required to train the individuals for the task, behavioral audiograms have been obtained for only a small percentage of individuals and species (Nachtigall et al., 2000); however, the AEP technique provides a means to rapidly test the hearing of untrained animals. To date, AEPs have been employed in obtaining audiograms on 13 species of cetaceans: *Tursiops truncatus* (Popov & Supin 1990a, 1990b), *Delphinus delphis* (Ridgway et al., 1981), *Phocoena phocoena* (Kastelein et al., 2002), *Pseudorca crassidens* (Dolphin, 1995; Yuen et al., 2005), *Grampus griseus* (Nachtigall et al., 2005), *Orcinus orca* (Szymanski et al., 1995), *Delphinapterus leucas* (Supin et al., 1993; Finneran et al., 2005), *Sotalia fluviatilis* (Supin et al., 1993), *Lipotes vexillifer* (Wang et al., 1992), *Inia geofrensis* (Popov & Supin 1990b), *Stenella coeruleoalba* (Kastelein et al., 2003), *Mesoplodon europaeus* (Cook et al., 2006), and *Neophocaena phocaenoides asiaeorientalis* (Popov et al., 2005). Importantly, the relationship between behavioural audiograms and those obtained with AEP techniques are being increasingly quantified, are comparable at mid-frequencies, and are similar at the low and high ends of an audiogram (Schlundt et al., 2005; Yuen et al., 2005; Finneran & Houser, 2006; Houser & Finneran, 2006). Only a relatively few species of marine mammals are in laboratory settings, which creates a great need for mobile AEP technology and techniques that allow investigations of species outside of the laboratory.

There are multiple reasons for the design of a portable system which measures AEPs. The most well-known reason addresses the growing concern about the increasing noise levels in our oceans. Anthropogenic noise sources, such as ships, geophysical surveys, oil and gas drilling, and military sonar, span a large range of frequencies and amplitudes (NRC, 2003). The extent to which these noise sources adversely impact marine mammals is poorly understood and is of special significance since the acoustic environment is critical for many aspects of ocean life. In order to create meaningful limitations on this noise production, especially around marine mammals, reliable and accurate hearing data must be obtained for as many individuals and species as possible. Second, due to their large size, baleen whales present a challenge to obtaining hearing data. Behavioral threshold measurements are impossible to obtain for these animals and, presently, none of these animals have been kept in captivity long enough to obtain rigorous hearing data. Stranding situations present our best opportunities to study rare species. Again, even in these situations, behavioral work is not reasonable for these individuals; however, the rapid and minimal participation requirement of the subject for AEP studies would be of great value.

To aid in this endeavor, we have created a portable system to measure the hearing capabilities of marine mammals. This system was designed to rapidly assess the underwater hearing capabilities of small odontocetes. The instrumentation has been, and still is, being optimized for use on a wide range of marine mammal species measured under field conditions, however, including pinnipeds and ursids.

Materials and Methods

Instrumentation

A laptop computer with a custom-made *LabView*® program generates an outgoing stimulus which is converted to an analog signal through a National Instruments DAQ 6062E card (Figure 1). This signal is then run through a custom-built attenuator box (with maximum attenuation of 70 dB in 1 dB increments). After the signal is attenuated, it is played through a calibrated transducer—ITC-1032 for 4 to 40 kHz, Reson TC4040 for 40 to 100 kHz, and Reson TC2130 for 100 to 300 kHz—giving the system a frequency capability of 4 to 300 kHz. The brainwaves of the subject are collected using two passive, custom-built suction-cup electrodes (Silastic RTV silicone rubber kit, Grass E5GH gold disc electrodes, RG 174/U coaxial cable, RCA connectors): one is the recording electrode and the other is the reference of biogenic noise. The collected brainwave potentials are then amplified 10,000 times using an Iso-dam amplifier (World Precision Instruments) and bandpass filtered using a Krohn-Hite Model 3103. The signal is then converted from analog to digital through the same NI DAQ card and visualized using the same *LabView*® program that generated the stimulus. Both the outgoing stimulus and the incoming, corresponding potentials are visualized using an OS – 310 M two-channel, digital oscilloscope (EZ Digital Co., Ltd.).

Procedure

The entire procedure revolves around the flexibility of our custom-written *LabView*® software. Depending upon the response of interest, the researcher can input a specific frequency into the program, which can generate tone pips, broadband clicks, or sinusoidally amplitude-modulated (SAM) stimuli. The stimulus update rate of the program can be varied up to 800 kHz, with this limitation being imposed by the capability of the NI DAQ card, which has allowed us to reliably generate stimuli as high as 256 kHz. The stimulus modulation rate can be varied between 100 Hz and 3 kHz, which can be accommodated by a sampling rate of 16 kHz on the incoming potentials. The stimulus length can

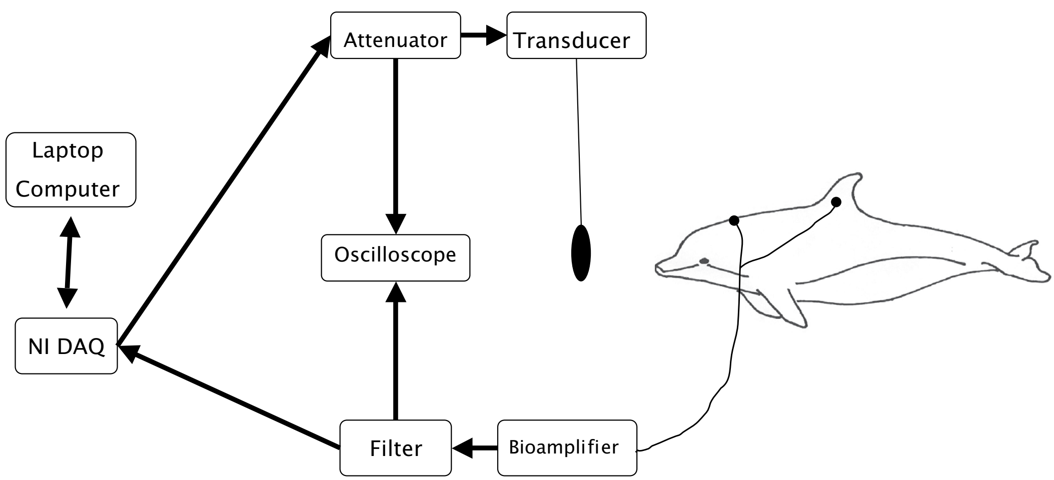

Figure 1. The equipment setup for the portable AEP system; the direction of the flow of data is denoted with arrows. A stimulus is generated by a custom *LabView*® program on a laptop computer; the signal is D/A converted, attenuated, and presented through an underwater transducer. The suction-cup electrodes acquire the evoked potentials, which are amplified, bandpass filtered, A/D converted, and saved by the same *LabView*® program that generated the outgoing stimulus. Both the outgoing stimuli and incoming potentials are monitored using a digital oscilloscope.

be as high as 20 ms, and the incoming stimulus can be analyzed up to 30 ms in order to visualize the onset and offset of the neuronal response. In addition, the software allows us to vary the amount of averages that we obtain for each record, which is important in order to be able to increase the signal-to-noise ratio. Increasing the average number correlates to an increase in the time it takes to measure one amplitude level of one frequency and can be reduced in time-critical measurements such as those on stranded or wild-caught animals.

The AEP procedure for obtaining audiograms in odontocetes consists of attaching two flexible, suction-cup electrodes onto the animal's dorsal side. The active electrode is placed approximately 6 to 8 cm behind the blowhole, where Popov & Supin (1990a, 1990b) have shown that the ABR has its highest amplitude, and the reference electrode is placed on or near the dorsal or pectoral fin. Most often a SAM signal modulated in the range of 875 Hz to 1.2 kHz (Supin & Popov, 1995) is used. Supin et al. (2001) and Supin (2006) have also shown that tone pips are sometimes more effective in evoking a maximal response. The stimulus is played through an underwater transducer, which has been calibrated in the measurement sound field at a distance from the subject (measured from the subject's external ear to the center of the projector) that allows far-field recording of the selected frequency (Supin et al., 2001). The electrodes acquire evoked potentials as well as background biogenic noise. The signal is then amplified, bandpass filtered around the modulation frequency (300 Hz to 3 kHz at 24 dB/oct), and averaged 1,000 times. This process is repeated over a range of frequencies and amplitudes to obtain a complete audiogram.

This system has the ability to be reconfigured in order to measure AEPs for a variety of marine mammal species. Hardware modifications would include in-air transducers and sound amplifiers as well as needle electrodes or laryngeal probe electrodes. The investigator would also have to take into account electrode placement and stimulus design for the acquisition of maximal responses as well as sedation regimes for the reduction of biogenic noise and for the safety of the researcher. For great whales, their immense size may necessitate the use of a laryngeal probe electrode in order to place the electrode much closer to the site of the neuronal source than our corresponding surface suction-cup electrode would allow. Stranded animal hearing measurement is possible in rehabilitation centers; to date, however, this system has not been tested in beach measurement conditions.

Data Analysis

After data collection is complete, the waveforms for each amplitude level of a specific frequency are compared. Figure 2 shows an example of the response of a bottlenose dolphin to clicks with a center frequency of 16 kHz and modulation rate of 1.125 kHz. The transfer function is seen after approximately 4 ms; however, our data collection window was set for 26 ms, so the offset response is not particularly clear. These waveforms are then fast Fourier transformed (FFT) using a 256-bin FFT in *Microsoft Excel*. The FFT bin size can be an issue of concern with short signals such as clicks and can underestimate the size of the frequency response; one will see a much higher response when analyzing longer signals such as SAM stimuli. To correct for these differences in FFT magnitude for different signal types, other FFT programs with variable bin sizes can be used or the magnitudes of the incoming potential waveform can be calculated and compared (for further analysis, see modulation rate transfer function [MRTF] publications such as Supin & Popov, 1995, and Mooney et al., 2006). For audiometric studies that do not vary the modulation rate between frequencies, the FFT of the waveforms can be analyzed in terms of their relative difference in magnitude as compared with the other amplitudes measured with the same stimulus modulation rate (Figure 3). The FFT values at the modulation rate are then plotted and a linear axis crossing regression line is calculated, which results in the threshold for that frequency (Figure 4).

Discussion

Portable systems are the future of AEP work on marine mammal species. Portability will greatly increase the likelihood of obtaining auditory studies on increasing numbers of individuals and species. These systems may also play a role as diagnostic hearing tools for marine mammal stranding networks as well as offer the possibility of gaining insight on the hearing capabilities of great whales.

The portable system outlined in this paper evolved from an earlier semi-portable system that is still in use in our laboratory. This original system, which Nachtigall and colleagues used to study the hearing of an infant Risso's dolphin (Nachtigall et al., 2005; Mooney et al., 2006), was spatially much larger, heavier, and more cumbersome than the current system. The previous system was run using a desktop computer setup. The *LabView*® program that was used with that system was not capable of designing its own waveform like the software of the current system, so the stimulus had to be designed with another graphing program prior to data collection. In addition, none of the previous components were battery capable, which necessitated the inclusion of very weighty devices

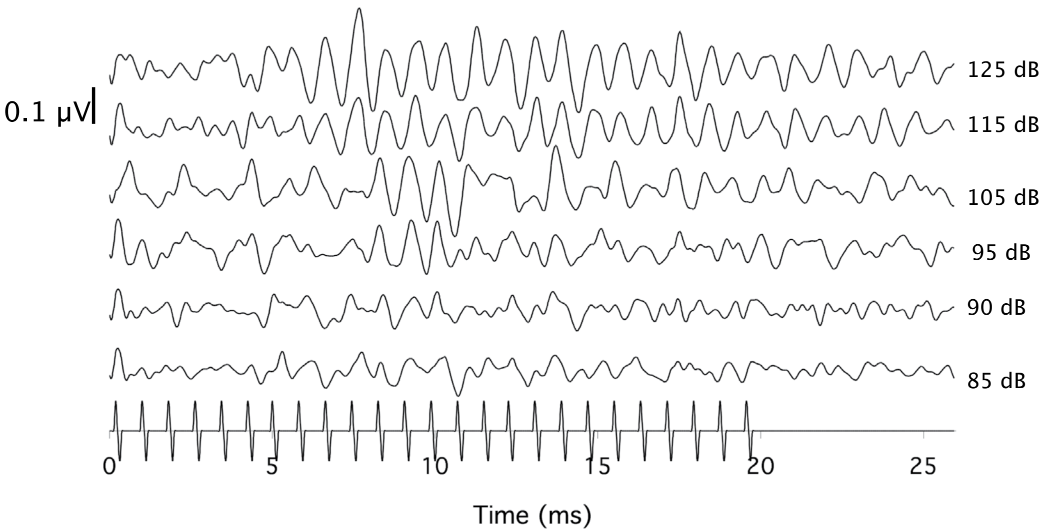

Figure 2. The evoked potential response waveforms of a male bottlenose dolphin to clicks with a center frequency of 16 kHz, a modulation rate of 1.125 kHz, and decreasing amplitudes, which are labeled to the side of each waveform.

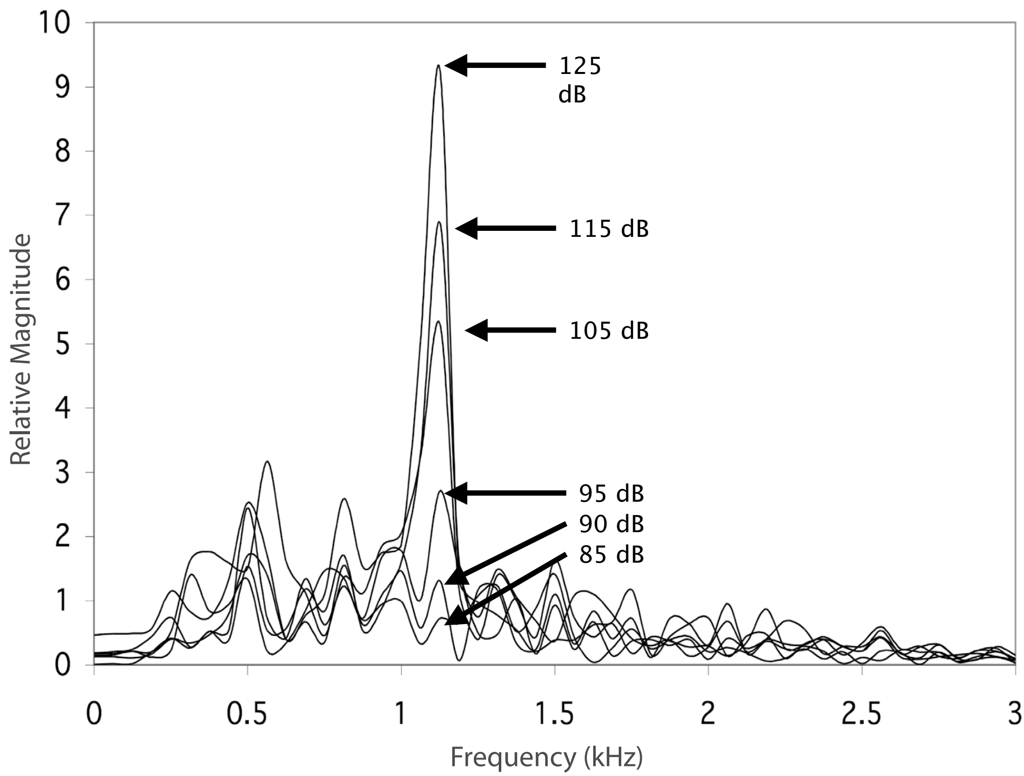

Figure 3. Fast Fourier transform (FFT) of the waveforms from Figure 2; the FFT response magnitudes are a relative measure of the acquired waveforms. Note the decreasing amplitude of the FFT with decreasing stimulus amplitude.

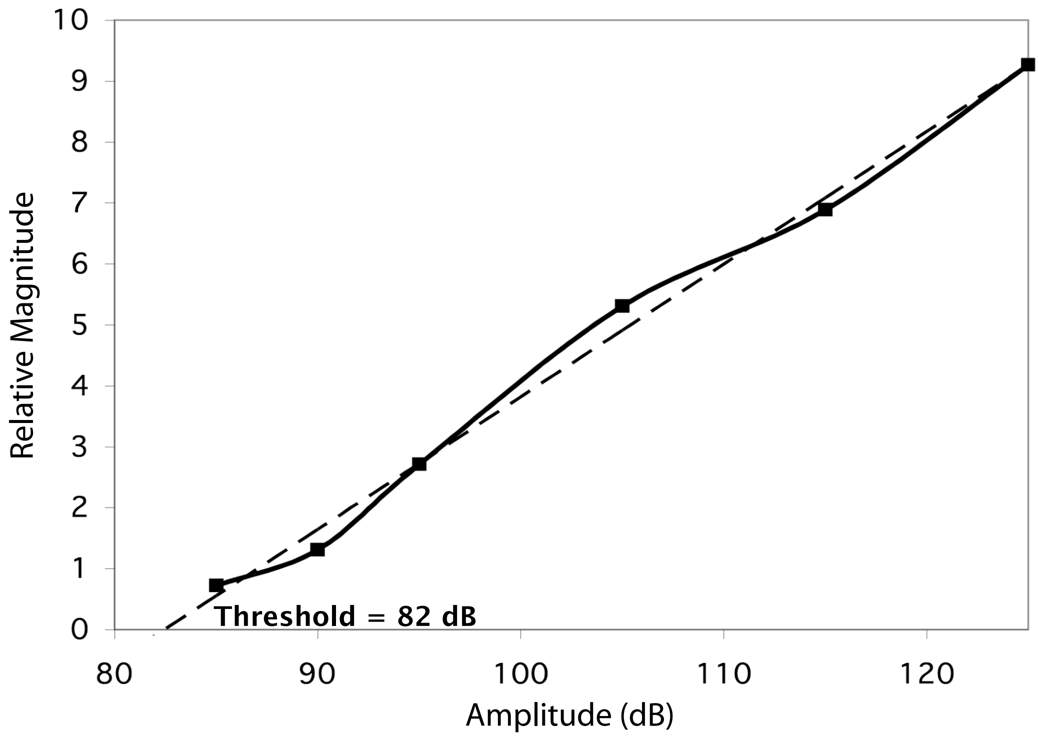

Figure 4. An example of threshold evaluation at 16 kHz for the FFT data of Figure 3; the solid line and points are actual measured data points, and the checked line is a linear regression line, which, when the zero point is calculated, assigns the threshold for this frequency at 82 dB.

such as power converters to be included with the gear. In total, the original system required four large Pelican (Model 1650) cases to transport, whereas the new system and its supporting gear can all be transported in one suitcase.

The current, portable AEP system is much smaller, lighter, and more modular than the old system. The laptop computer, oscilloscope, and bioamplifier are battery powered, a feature which greatly decreases the ground power needed to run the entire system. In addition to increased battery capability, the system is increasingly modular, which is advantageous for multiple reasons. First, each component can be individually checked and calibrated for accuracy. Second, components that are more prone to electrical noise interference, such as the bioamplifier, can be placed a distance away from the other components such as the computer, filter, etc., thereby decreasing the chance for interference. Finally, in a field situation, it is never known how much working room will be available. These pieces of equipment can be extended away from each other in order to increase the distance this system can reach to the animal being tested. This system was used to acquire hearing data on bottlenose dolphins at Kolmården Djurpark, Sweden. The trainers stationed the animal from a floating floor in a concrete, indoor pool with the nearest dry deck space with which to store the laptop being over 15 m away. The modular system was able to span the distance between the computer and suction cups without the suction-cup electrodes having to be of a length that the signal is attenuated.

In addition to our system, there are a small number of laboratories working with portable and semi-portable marine mammal AEP systems (Mann et al., 2005; Finneran & Houser, 2006). All of these systems have the same general design but with components obtained, and in some cases, highly modified, from various manufacturers. There are also complete AEP systems commercially available: the MASTER system by Bio-Logic Systems Corporation as well as Tucker Davis Technologies (TDT). The MASTER system is based on human hearing research practices and does not have the frequency and signal range to accommodate testing on marine mammal species. The TDT system is much more promising as a research tool for some marine mammal species. To date, their system has a maximum frequency range up to 115 kHz, which could accomplish the measurement of full audiograms on some of the lower-frequency species such as pinnipeds, but it would not be able to measure the full high-frequency range of the porpoises and other high frequency species.

Despite differences in the hardware components and software capabilities of the different marine mammal AEP groups, we have had extensive discussions with members of these teams on how to optimize the materials and methods. It is because of this cooperation between these different groups that, rather than having a static system, this system is constantly evolving. In addition to increasing the performance capability of each individual hardware component, we are improving the software capability. We are working to increase the flexibility of stimulus types so that we are able to better tailor the stimulus for the maximum response magnitude for each individual species. With the increasing range of frequencies and amplitudes, we will be focused on gathering as much AEP data on as many individuals of as many species as possible. With the acquisition of data for new individuals and species, we can begin to explore the normal variations in evoked potential latencies and amplitudes. This information would facilitate the use of AEP techniques not only as research tools but as diagnostic tools to explore the health of marine mammal hearing.

Acknowledgments

The authors thank the rest of the members of the Marine Mammal Research Program for their help and support on the creation of this system. We would especially like to thank Dr. Whitlow Au for help with hardware issues and Stuart Ibsen for software support. A very special thank you is given to Heidi Taylor for her artwork of a *Tursiops*. We would also like to thank the editor and two anonymous reviewers that took the time to add their valuable comments to the editing process.

Literature Cited

Cook, M. L. H., Varela, R. A., Goldstein, J. D., McCulloch, S. D., Bossart, G. D., Finneran, J. J., et al. (2006). Beaked whale auditory evoked potential hearing measurements. *Journal of Comparative Physiology A*, *192*, 489-495.

Dolphin, W. F. (1995). Steady-state auditory-evoked potentials in three cetacean species elicited using amplitude-modulated stimuli. In R. A. Kastelein, J. A. Thomas, & P. E. Nachtigall (Eds.), *Sensory systems of aquatic mammals* (pp. 25-47). Woerden, The Netherlands: DeSpil.

Finneran, J. J., & Houser, D. S. (2006). Comparisons of in-air evoked potential and underwater behavioral hearing thresholds in four bottlenose dolphins (*Tursiops truncatus*). *Journal of the Acoustical Society of America*, *119*(5), 3181-3192.

Finneran, J. J., Dear, R., Carder, D. A., Belting, T., McBain, J., Dalton, L., et al. (2005). Pure tone audiograms and possible antibiotic drug-induced hearing loss in the white whale (*Delphinapterus leucas*). *Journal of the Acoustical Society of America*, *117*, 3936-3943.

Hall, J. W., III. (1992). *Handbook of auditory evoked responses*. Boston: Allyn & Bacon. 886 pp.

Houser, D. S., & Finneran, J. J. (2006). A comparison of underwater hearing sensitivity in bottlenose dolphins (*Tursiops truncatus*) determined by electrophysiological and behavioral methods. *Journal of the Acoustical Society of America*, *120*(3), 1713-1722.

Jewett, D. L., Romano, M. N., & Williston, J. S. (1970). Human auditory evoked potentials: Possible brainstem components detected in the scalp. *Science*, *167*, 1517-1518.

Jewett, D. L., Romano, M. N., & Williston, J. S. (1971). Auditory-evoked far fields averaged from the scalp of humans. *Brain*, *94*, 681-696.

Kastelein, R. A., Bunskoek, P., & Hagedoorn, M. (2002). Audiogram of a harbor porpoise (*Phocoena phocoena*) measured with narrow-band frequency-modulated signals. *Journal of the Acoustical Society of America*, *112*, 334-344.

Kastelein, R. A., Hagedoorn, M., & de Haan, D. (2003). Audiogram of a striped dolphin (*Stenella coeruleoalba*). *Journal of the Acoustical Society of America*, *113*, 1130-1144.

Mann, D. A., Colbert, D. E., Gaspard, J. C., Caper, B. M., Cook, M. L. H., Reep, R. L., et al. (2005). Temporal resolution of the Florida manatee (*Trichechus manatus latirostris*) auditory system. *Journal of Comparative Physiology A*, *191*, 903-908.

Mooney, T. A., Nachtigall, P. E., & Yuen, M. M. L. (2006). Temporal resolution of the Risso's dolphin, *Grampus griseus*, auditory system. *Journal of Comparative Physiology A*, *192*, 373-380.

Nachtigall, P. E., Lemonds, D. W., & Roitblat, H. L. (2000). Psychoacoustic studies of dolphin and whale hearing. In W. W. L. Au, A. N. Popper, & R. R. Fay (Eds.), *Hearing by whales and dolphins* (pp. 330-363). New York: Springer-Verlag.

Nachtigall, P. E., Yuen, M. M. L., Mooney, T. A., & Taylor, K. A. (2005). Hearing measurements from a stranded infant Risso's dolphin (*Grampus griseus*). *Journal of Experimental Biology*, *208*, 4181-4188.

National Research Council (NRC). (2003). *Ocean noise and marine mammals.* Washington, DC: National Academy of Science. 204 pp.

Popov, V. V., & Supin, A. Ya. (1990a). Auditory brainstem responses in characterization of dolphin hearing. *Journal of Comparative Physiology*, *166*, 385-393.

Popov, V. V., & Supin, A. Ya. (1990b). Electrophysiological studies of hearing in some cetaceans and a manatee. In J. A. Thomas & R. A. Kastelein (Eds.), *Sensory abilities of cetaceans* (pp. 405-415). New York: Plenum Press.

Popov, V. V., Supin, A. Ya., Ding, W., Wang, K., Xiao, J., & Li, S. (2005). Evoked potential audiogram of the

Yangtze finless porpoise *Neophocaena phocaenoides asiaeorientalis*. *Journal of the Acoustical Society of America*, *117*(5), 2728-2731.

Ridgway, S. H., Bullock, T. H., Carder, D. A., Seeley, R. L., Woods, D., & Galambos, R. (1981). Auditory brainstem response in dolphins. *Proceedings of the National Academy of Science USA*, *78*, 1943-1947.

Schlundt, C. E., Finneran, J. J., & Houser, D. S. (2005). Simultaneous use of auditory evoked potential (AEP) and behavioral methods to determine hearing abilities in a bottlenose dolphin. *Proceeding of the Sixteenth Biennial Conference on the Biology of Marine Mammals*, San Diego, CA. 330 pp.

Sohmer, H., & Feinmesser, M. (1967). Cochlear action potentials recorded from the external ear in man. *Annals of Otology Rhinology Otolaryngology*, *76*, 427-435.

Supin, A. Ya. (2006). Development of evoked-potential audiometry in odontocetes. *Journal of the Acoustical Society of America*, *120*(5, Pt. 2), 3226.

Supin, A. Ya., & Popov, V. V. (1995). Envelope-following response and modulation transfer function in the dolphin's auditory system. *Hearing Research*, *92*, 38-45.

Supin, A. Ya., Popov, V. V., & Klishin, V. O. (1993). ABR frequency tuning curves in dolphins. *Journal of Comparative Physiology*, *173*, 599-656.

Supin, A. Ya., Popov, V. V., & Mass, A. M. (2001). *The sensory physiology of aquatic mammals.* Boston: Kluwer Academic Publishers. 332 pp.

Szymanski, M. D., Bain, D. E., & Henry, K. R. (1995). Auditory evoked potentials of killer whales (*Orcinus orca*). In R. A. Kastelein, J. A. Thomas, & P. E. Nachtigall (Eds.), *Sensory systems of aquatic mammals* (pp. 1-9). Woerden, The Netherlands: De Spil.

Wang, D., Wang, K., Xiao, Y., & Sheng, G. (1992). Auditory sensitivity of a Chinese River dolphin, *Lipotes vexillifer*. In J. A. Thomas, R. A. Kastelein, & A. Ya. Supin (Eds.), *Marine mammal sensory systems* (pp. 213-221). New York: Plenum Press.

Yuen, M. M. L., Nachtigall, P. E., & Breese, M. (2005). Behavioral and auditory evoked potential audiograms of a false killer whale (*Pseudorca crassidens*). *Journal of the Acoustical Society of America*, *118*(4), 2688-2695.