Hearing and Auditory Evoked Potential Methods Applied to Odontocete Cetaceans

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Abstract
Auditory evoked potential (AEP) procedures have been increasingly used to measure hearing processes in aquatic mammals. They have been demonstrated to be useful in measuring the audiograms of stranded animals like infant sperm whales (Physeter macrocephalus) and Risso’s dolphins (Grampus griseus). Modulation rate transfer functions (MRTF) demonstrating appropriate stimulus presentation rates are usually measured prior to recording audiograms with odontocetes. Measures comparing behavioral and AEP audiograms with the same animals have generally shown good correspondence between data gathered using the two procedures. AEPs and acoustic brainstem responses (ABRs) also have been used to measure hearing while an animal is actively echolocating. This technique of measuring the animal’s ability to hear its own outgoing signals, as well as the returning echoes, allows experimenters to develop a new understanding of the processes underlying echolocation.

Key Words: auditory evoked potential, acoustic brainstem response, modulation rate transfer function, envelope-following response, sperm whale, Physeter macrocephalus, Risso’s dolphin, Grampus griseus

Introduction
Marine mammal hearing traditionally has been measured using behavioral techniques in which the animal was trained to respond when a sound was heard and to not respond when no sound was presented. This is a very reliable way to measure hearing, and the experimenter can be assured that if the proper controls are applied, the animal is truly hearing the sound that is presented. Usually, pure tones of one particular frequency are presented at varying levels, and the point at which the animal just barely hears the tone is termed the threshold. An audiogram is created by plotting the thresholds of all frequencies across a wide range of frequencies on a single graph. Typically, mammalian audiograms are U-shaped, indicating that the animal has elevated thresholds at low frequencies, followed by lower thresholds during peak sensitivity, and then followed by sharply elevated thresholds and decreased sensitivity at very high frequencies.

While behavioral thresholds are excellent ways of measuring hearing, they also have considerable constraints. They require that the animal be well trained to (1) remain in a fixed position in order to keep sound levels constant during hearing, (2) report the presence of sound if it is heard, (3) not report a sound when it is not heard, and (4) endure the potentially considerable frustration when the sounds are very quiet and the task is very difficult. This training usually requires that the animal reside in a laboratory or other facility that houses animals. The training and data collection for a naïve marine mammal also take a considerable amount of time. It may take two years to train and test the hearing of a naïve dolphin. This requirement for maintaining and training the animal limits both the species and the numbers of animals from which hearing data are collected (Nachtigall et al., 2000).

Electrophysiological Methods for Hearing Thresholds
Behaviorally measured audiograms will remain as the primary method for measuring hearing because they actually depend on the report of an animal’s experience of hearing something. Recent developments using auditory brainstem responses (ABRs) have added a new procedure for rapidly measuring hearing, however, especially in situations where behavioral tasks are not possible or practical. An ABR allows the measurement of what an animal hears through recording and measuring electrical impulses from the brain that synchronously occur in response to sound. Two
developments in the measurement techniques for recording ABRs made them satisfactory for use with marine mammals: (1) they can be collected from the surface of the skin using human electroencephalogram (EEG) sensors placed within soft latex rubber suction cups for cetaceans, and (2) acoustic signals comparable in length to behavioral audiogram signals can be presented, allowing ABRs to be measured using envelope following responses (EFRs) to amplitude-modulated acoustic stimuli (Dolphin et al., 1995; Supin & Popov, 1995).

Auditory evoked potential (AEP) measurements of hearing do not require that the animal be trained. Most dolphins and small whales that strand themselves are very passive and easily handled, and most of those undergoing rehabilitation can simply be held during an AEP hearing examination. We had the opportunity to test the hearing of two infant odontocetes of rare species: a sperm whale (Physeter macrocephalus) and a Risso’s dolphin (Grampus griseus) (Nachtigall et al., 2005). The infant female sperm whale stranded in 2001 off the Kona coast of the Big Island of Hawaii. After some negotiation with the U.S. National Oceanic & Atmospheric Administration (NOAA) stranding network coordinator, and under the auspices of the permit of Dr. Sam Ridgway, Dr. Alexander Supin began the AEP measurements of the whale. EFRs are the brain signals that follow the acoustic signals that are placed into the water. If a signal is amplitude-modulated at 1,000 times per second, the odontocete brain waves usually follow that modulation rate (Dolphin et al., 1995; Supin & Popov, 1995). The first thing necessary when examining a new species is to measure how well their brain waves follow the modulation rates of stimuli presented to them. The resulting data of the quantified ability to follow is plotted as a function of the modulation rate and is termed the modulation rate transfer function (MRTF); therefore, the first assessment conducted with the infant sperm whale was to attempt to determine an MRTF.

Suction cups containing small gold electrodes like those used for human EEG analysis were placed over the sperm whale’s cranial area on the surface of its skin and on its back (Figure 1). Unfortunately, as with many stranded animals, necessary and agreed veterinary care overtook the priority for scientific data, and we were unable to complete the MRTF for the sperm whale infant.

Work with the infant Risso’s dolphin proved more successful, however. We had the opportunity to measure the hearing of a stranded infant Risso’s dolphin at the Zoomarine facility in Albufeira, Portugal. This infant stranded along with other individuals of unknown species along the Algarve Coast and was brought to the rehabilitation facility of Pedro Lavia and Élio Vicente. Given that the animal was listing to one side, there was concern for the animal’s vestibular and ear function; we were invited to examine the animal’s hearing (Nachtigall et al., 2005). As noted, the first step before measuring an animal’s audiogram with an AEP procedure is to determine how well it hears stimuli that are amplitude-modulated at various rates—in other words, to determine the MRTF. We measured the MRTF of the Risso’s dolphin with signals varying from 100 to 2,000 times per second (Mooney et al., 2006). We presented broadband clicks containing energy from 1 to 40 kHz designed as a rectangle function that was 50 μs in duration. The broadband nature of the stimuli ensured that the animal’s presumed high-frequency hearing and the click bandwidth would overlap.

The animal’s responses were recorded using two standard 10-mm gold EEG sensor electrodes in two latex suction cups that were placed on the surface of the subject’s skin. Passive conductivity of the animal’s AEPs from the skin surface to the electrode was enhanced by standard human EEG gel. One suction cup was embedded within the recording electrode and was placed 3 to 4 cm behind the dolphin’s blowhole and off to the right (i.e., over the animal’s brain). The second suction cup contained the reference electrode and was placed on the back of the animal near its dorsal fin. The animal rested at the surface with most of its head and lower jaw under water to receive sound input through the major tissue routes to the ears (Norris, 1968; Möhl et al., 1999; Ketten, 2000), but with the suction cups in the air. Retaining the suction cups in the air enhanced the electrical signal.

An ABR refers to a response to a single ping or tone pip, as opposed to an EFR, which refers to a response to a longer stimulus or series of ABR events. The EFR measurement not only allows the measurement of the following response, it also allows for the measurement of a longer tone.

Figure 1. Infant sperm whale with suction-cup sensors attached while attempting to evaluate MRTF for AEPs
stimulus, thereby using rms values that are more directly comparable to longer stimulus pure tones of traditional behavioral techniques that are normally presented in rms values. Thus, EFR measurements are easily and directly comparable to behaviorally determined thresholds, more so than individual tone pip ABRs.

The actual signals to this animal were 20 ms long, made up of a series of clicks of various lengths. Each of these 20-ms signals was played at least 1,000 times. The EFRs from the animal were averaged and fast Fourier transformed (FFT), resulting with a peak at each of the varying frequencies determined and plotted as the MRTF (Figure 2) in terms of relative microvolts. These data clearly show that, like other odontocetes, the infant Risso’s dolphin readily followed the pattern of the signal up to 1,000 times per second. This finding is important in that it allows a rapid collection of Risso’s dolphin hearing data because carrier frequencies can be modulated up to 1,000 times per second and the pattern can be examined. This ability to follow rapid signals is likely built into the acoustic system because of the odontocete’s ability to echolocate (Supin et al., 2001).

Once the MRTF was determined, the actual hearing of the infant Risso’s dolphin could be examined. Because this was the first hearing examination of a neonate Risso’s dolphin, sounds were presented to the animal based on threshold levels of a previously measured adult Risso’s dolphin (Nachtigall et al., 1995) and a bottlenose dolphin (*Tursiops truncatus*) (Johnson, 1968). We started at levels 20 to 30 dB above the lowest threshold levels of the preceding audiograms. Eighteen carrier-frequencies were tested, ranging from 4 to 150 kHz. The following frequencies were first tested based on the previous Risso’s audiogram: 4.0, 5.6, 8.0, 11.2, 16.0, 22.5, 32.0, 40.0, 50.0, 64.0, 76.0, 80.0, 90.0, 100.0, and 110.0 kHz. The animal heard very well at the highest of these frequencies (110.0 kHz), and measurements of an additional three frequencies (108.0, 128.0, and 150.0 kHz) were successively added. The beginning levels for these three frequencies were determined by starting 20 to 30 dB above the previously obtained threshold. The animal’s responses to the sounds were monitored.

The amplitudes of the amplitude-modulated tone-bursts were reduced in 5- to 10-dB steps until the AEP responses to the sounds could no longer be distinguished from the background noise. The amount that the stimulus was lowered in each step was varied according to the response observed by the experimenter as the data were gathered. An average of nine intensity levels was presented for each of the 18 different frequencies. The carrier-frequency sounds were initially calibrated at each frequency tested using continuous pure tones measured at the position of the animal’s head. The received peak-to-peak levels (V) of the stimuli were measured and used to calculate pe rms (V) and received sound pressure level (SPL). These values were taken as the received level of each stimulus frequency. As the amplitude-modulated stimuli were presented, the values were converted to rms (V) to determine the equivalent received levels.

The response to each modulated carrier-frequency was examined at each intensity level by

![Figure 2](image)

**Figure 2.** Modulation rate transfer function for infant Risso’s dolphin; relative amplitude of record response as a function of repetition rate of 50 μs broadband clicks (Mooney et al., 2006).

![Figure 3](image)

**Figure 3.** Evoked potential records of responses following amplitude-modulated stimuli at various levels ranging from 55 to 80 dB.
looking at the evoked potential record, consisting of at least 1,000 evoked responses. Each presentation was 20 ms long followed by a 30-ms quiet interval (Figure 3). FFTs were calculated for a 16-ms window (also shown in Figure 3) of the average evoked response recorded at each intensity level for each frequency in order to quantitatively estimate the animal’s hearing threshold. Each of these windows contained a whole number of response cycles to the amplitude-modulated stimulus. The FFT peak level was determined for each of the stimulus intensity levels. A larger EFR response was reflected as a higher peak value. The peak FFT amplitude at the 1,000 Hz modulation rate was used to estimate the magnitude of the response evoked by the modulated stimulus.

To determine the animal’s threshold for each frequency tested, the FFT peak at each stimulus intensity level was plotted as response intensity against the SPL of the stimulus in the same way (Figure 4). Linear regression calculated on the points was extended to the zero-crossing point. With the stimulus SPL value at the zero response, it was possible to estimate the threshold for each of the frequencies presented to the animal.

The quiet environment of the concrete tank, without the extraneous natural noises found in a normal ambient environment (Nachtigall et al., 1995), provided an excellent opportunity to obtain threshold values without background noise interference. The animal’s averaged evoked responses could be clearly observed as the data were gathered. There was normally a 4- to 5-ms lag on both the onset and offset of the tone-burst stimulus. This served as a predictable electrophysiological feature, demonstrating that the brainwave recording occurred in direct response to the acoustic stimulus. When stimulus intensities were high, EFRs were discernable well above the noise level (as previously shown in Figure 3). As the measurements approached the auditory threshold levels, the decreasing EFR magnitudes reflected the synchronously decreasing levels of the stimuli.

The hearing threshold for each of the carrier frequencies was determined in a like manner, with thresholds calculated as the stimulus level predicted to generate a response amplitude of zero. Results of the threshold calculations are depicted as an audiogram (Figure 5), along with the results of the only other audiogram of a Risso’s dolphin, which was collected from an older animal (Nachtigall et al., 1995, 2005). The infant animal showed a wide range of best sensitivity with hearing thresholds lower than 60 dB between 22.5 and 90.0 kHz. The lowest thresholds were 50 dB or lower at three of the measured frequencies (32.0, 64.0, and 90.0 kHz).

The AEP audiogram’s general shape was a typical mammalian U-shape (Figure 5). At high frequencies, the slope of thresholds increased steeply beyond 90 kHz at a rate of 95 dB/octave. Below 32 kHz, the slope of increasing thresholds was more gradual, at 16.4 dB/octave. Poorest sensitivity was measured at the very low and very high frequencies—100.3 dB at 4.0 kHz and 116.9 dB at 150.0 kHz, respectively. Further details of this experiment and a comparison between the hearing of the infant Risso’s dolphin and the older adult Risso’s dolphin may be found in Nachtigall et al. (2005).

Comparison of AEP and Behavior
While it has been noted for a considerable amount of time that neuroelectric events increase in magnitude as sensory stimuli increase (Stevens, 1970),
early direct magnitude auditory demonstrations were limited to cochlear microphonics. The advent of the development of EFR procedures for hearing measurement allowed for the possibility of a direct comparison between behavioral and AEP measures of hearing because both could be based on stimuli with rms measures (Dolphin et al., 1995; Supin & Popov, 1995). Yuen et al. (2005) were interested in how audiograms directly compared when the hearing of the false killer whale (*Pseudorca crassidens*) was measured with both behavioral and EFR techniques. The whale’s hearing had been measured three times during a three-year period using EFRs. Each of the animal’s thresholds had been determined in a very similar manner to that described above for the neonate Risso’s dolphin. Sinusoidally amplitude modulated (SAM) stimuli were presented at various levels, and the averaged EFRs were recorded to those stimuli (Figure 6). The peaks of the FFTs of the various levels were analyzed with a linear regression, and the point at which the line would have crossed zero was calculated as the threshold for each frequency. These thresholds were then plotted by amplitude to create the audiogram. This audiogram was then compared to an audiogram measured behaviorally.

The behavioral audiogram data were collected with the requirement that the animal report the acoustic stimulus if it was heard. Instead of a 20-ms SAM carrier frequency played at least 1,000 times, the animal was given the same frequency played as a pure tone for 3 s. If the animal heard the tone, she responded by pressing a paddle, and if she did not, she just remained stationed in the hoop; complete details are available (Yuen et al., 2005). The comparison of these two audiograms is presented in Figure 7. Generally, the threshold data from the two audiograms are quite similar and follow one another, with the behavioral audiogram showing an average of about 10 dB better sensitivity. The better sensitivity measured behaviorally might have been primarily due to the fact that the EFR stimulus was only 20 ms long while the behavioral stimulus was 3 s. In general, animals listening for very short signals do not hear them, as well as they do longer signals (Johnson, 1968).

The finding of the comparability of the EFR and behavioral techniques was very recently verified with work by Houser & Finneran (2006) using a number of bottlenose dolphins in a variety of situations. Stimuli were presented in tanks on shore and in a noisy bay, with transducers in a free field and also from jawphones to animals laying on mats in air. Houser & Finneran found that the estimates of hearing sensitivity of delphinids from recordings of EFR show similar degrees of accuracy and precision relative to behavioral thresholds, regardless of the exact methodology used to deliver the stimulus.

**Measuring Hearing During Echolocation**

Echolocation is described as the emission of a sound followed by listening to the echo of...
that sound. While there has been a great deal of emphasis on the description of the outgoing signals (Au, 1993), there has been very little work describing an odontocete’s ability to listen to the echoes of their own outgoing clicks. The reason for the sparse research emphasis on listening during echolocation in dolphins and whales is that there has been no technique for measuring hearing during echolocation. Indeed, echolocating dolphins and toothed whales hear weak echo signals very shortly after emitting intense echolocation clicks, but until EAPs were measured during an active echolocation task (Supin et al., 2003, 2004, 2005, 2006a), nothing was known about how well an odontocete heard its outgoing echolocation click or its corresponding echo. The measurement of hearing during echolocation allows for the development of a new understanding of echolocation processes.

In the initial work by Supin et al. (2003), a false killer whale (Pseudorca crassidens) was trained to report the presence or absence of an aluminum cylinder, a technique to ensure that the animal was echolocating. The whale stationed within a hoop while wearing the same gold suction-cup electrodes used for basic audiogram measurements. The animal’s detection and reporting of the presence or absence of the cylinder via echolocation remained above 97% correct, while ABR recordings were made of her responses to both her own outgoing signals and the echoes received from the target. The ABR recordings were triggered by the onset of the animal’s own clicks. The time from the click onset to the animal’s ears, and from the target returning to the ears, was calculated and “windowed” to precisely determine where in time to locate the appropriate ABRs. In the first experiment, although the click was of course much louder than the echo, the ABRs were of comparable amplitude. The animal heard the outgoing click and the return echo at about the same levels. Perhaps the most important thing about this experiment was that it demonstrated that ABR techniques could be used to measure hearing during particular events of an animal’s echolocation process.

The next experiment measuring hearing events during echolocation examined how well the whale could hear echoes as they changed in amplitude due to changes in the target’s distance from the whale (Supin et al., 2004). The whale continued to echolocate and report the presence or absence of a small aluminum cylinder while ABRs were recorded in response to the animal’s own outgoing clicks and echoes. Interestingly, even though varying the target distance caused changes in the echo intensity by up to 36 dB, the amplitude of the echo-related AEP was independent of distance. The animal apparently heard the target echoes equally well even though there was a 36 dB difference between them. Once again, even though there was a nearly -64 dB difference relative to the transmitted pulse in front of the head, the animal’s AEPs to the outgoing pulse and the target echoes were also nearly equal.

This brought up some interesting questions. How could the whale’s auditory system handle signals of such diverse intensities at essentially the same levels? Was there some sort of automatic gain control going on, and if so, what were the mechanisms of control?

In the third experiment, the paradigm was made even more challenging (Supin et al., 2005). Cylinders of different lengths were presented to the whale. Longer cylinders have greater target strength values than shorter cylinders while the other echo characteristics remain relatively similar. Four cylinders of -40, -34, -28, and -22 dB were presented at distances of 1.5, 3.0, and 6.0 m; either decreasing target strength or increasing distance will make the target echoes quieter. The difference between target echo returns of the closest, largest target and the furthest, smallest target was 42 dB. Once again, there was no difference in the outgoing pulse amplitudes produced by the whale in any of the target conditions. While other field investigators (Rasmussen et al., 2002; Au & Benoit-Bird, 2004) showed increases in pulse amplitudes with distance as a gain control mechanism, this was not evident in any of these studies. What became evident, however, was a change in the ABRs of the outgoing signal that was dependent on the targets. When the smallest targets were presented, the AEP amplitudes to the outgoing signals increased nearly three times when compared to the largest targets that were presented, and all the while, the sensitivity to the echo returns were the same for both targets. Thus, we were able to hypothesize that in our experiments, the whale was capable of changing its hearing sensitivity. It seems as though the whale has an automatic gain control mechanism based on its ability to change its hearing sensitivity during echolocation.

Further AEP echolocation hearing work investigated the similarity between hearing levels of the animal’s own loud, outgoing pulse and extrinsically produced pulses of similar levels. In other words, how well did the animal hear its own outgoing click compared to a similar click produced directly in front of it? This was accomplished by comparing AEP peak-to-peak amplitude dependence on the sound click intensities of the whale’s own outgoing clicks to similar clicks produced directly in front of the whale (Supin et al., 2006). Once again, the animal was presented with an echolocation task of detecting and reporting cylinders and the corresponding AEPs were recorded.
In the second phase of the experiment, the animal simply stationed within the hoop while simulated false killer whale echolocation clicks of varying intensities were presented and the AEP recordings were collected. Interestingly, depending on whether targets were present or absent, the sensitivity of the whale’s hearing to its own transmitted biosonar pulses was 30 to 45 dB lower than when the simulated echolocation clicks were presented in front of it. The fact that there was a difference in sensitivity to biosonar pulses depending on whether targets were present or absent indicates some sort of control mechanism for hearing during echolocation. The fact that there is a lower sensitivity to the animal’s own transmitted pulses than to sounds arriving from the outside shows that there are adaptations to control the intensity of the hearing of the outgoing pulse.

Most auditory systems demonstrate forward-masking effects. If a loud sound is heard, there is a short refractory period before the system is fully functional. Generally, the louder the first sound, the longer it takes for the auditory system to fully hear the second sound. Recent efforts by Supin et al. (2006b) examined the whale’s ability to hear two pulses in a forward-masking paradigm that modeled the outgoing and received transmitted pulses and returned echo during echolocation. Following similar two-pulse studies of bottlenose dolphins (Supin et al., 2001), the ABRs of the whale were measured with two pulses of varying intensities and intervals between the clicks.

The false killer whale data were similar to those of the bottlenose dolphin. This two-pulse simulation of echolocation showed that partial masking of the echo by the preceding emitted click may explain the independence of echo-response amplitude from target distances found in some of the previous work. The distance range where this mechanism is effective depends on the emitted click level, however. In other words, the higher the level, the greater the range.

Overall Value of AEPs for Odontocete Hearing Measurement

While AEPs may not be measuring exactly what is perceived or experienced during hearing (Stevens, 1970), the work by Yuen et al. (2005) and Houser & Finneran (2006) indicated that AEP measurements can be used as audiometric measurements. This has a significant value when attempting to measure the hearing of large, valuable, hard-to-maintain marine mammals. Rice (1998) lists 83 cetacean species, and since that publication, at least one new species has been added (Dalebout et al., 2002) and likely more will follow. Of the at least 84 cetacean species, only 11 species of odontocetes have measured audiograms (Yuen et al., 2005), with no mysticete audiograms yet completed. AEP measurement for audiograms is a valuable new technique for investigating the basic hearing of cetaceans.

This technique is valuable because it is fast, portable, and accurate when used by well-trained individuals. It can be used with stranded and untrained animals in rehabilitation facilities (Nachtigall et al., 2005), under water (André et al., 2003; Cook et al., 2006), and on mats in air with sounds presented with jawphones (Houser & Finneran, 2006). While a behavioral audiogram requires training, a captive animal facility, and years of effort, AEP audiograms can be completed in 90 minutes or less of experimental time with the animal. The rapid determination of thresholds assists in other hearing tasks. Temporary threshold shifts can be determined more accurately because practiced single AEP thresholds can be gathered in less than five minutes before significant recovery from initial sound exposure occurs (Nachtigall et al., 2004).

The AEP procedure is also adaptable to new techniques to address new questions that cannot be answered in other ways. The ability to reliably and accurately measure what an animal hears of its outgoing echolocation click and returning echo while it is echolocating opens an exciting new way to explore and understand a variety of auditory processes underlying the odontocete’s ability to echolocate. This AEP procedure will be available to new situations and to answer new questions about odontocete hearing and echolocation.

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