Behavioral and auditory evoked potential audiograms of a false killer whale (*Pseudorca crassidens*)

Michelle M. L. Yuen, a) Paul E. Nachtigall, and Marlee Breese  
*Marine Mammal Research Program, Hawaii Institute of Marine Biology, University of Hawaii, Kailua, Hawaii 96744-1106*

Alexander Ya. Supin  
*Institute of Ecology and Evolution, Russian Academy of Sciences, 33 Leninsky Prospekt, 119071 Moscow, Russia*

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Behavioral and auditory evoked potential (AEP) audiograms of a false killer whale were measured using the same subject and experimental conditions. The objective was to compare and assess the correspondence of auditory thresholds collected by behavioral and electrophysiological techniques. Behavioral audiograms used 3-s pure-tone stimuli from 4 to 45 kHz, and were conducted with a go/no-go modified staircase procedure. AEP audiograms used 20-ms sinusoidally amplitude-modulated tone bursts from 4 to 45 kHz, and the electrophysiological responses were received through gold disc electrodes in rubber suction cups. The behavioral data were reliable and repeatable, with the region of best sensitivity between 16 and 24 kHz and peak sensitivity at 20 kHz. The AEP audiograms produced thresholds that were also consistent over time, with range of best sensitivity from 16 to 22.5 kHz and peak sensitivity at 22.5 kHz. Behavioral thresholds were always lower than AEP thresholds. However, AEP audiograms were completed in a shorter amount of time with minimum participation from the animal. These data indicated that behavioral and AEP techniques can be used successfully and interchangeably to measure cetacean hearing sensitivity.


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I. INTRODUCTION

In 1966, Johnson measured the first complete audiogram of an individual Atlantic bottlenose dolphin, *Tursiops truncatus* (Johnson, 1966). The results indicated that the animal’s range of sensitivity to pure tones was from 75 Hz to 150 kHz, an exceptionally wide range covering more than 100 kHz and 11 octaves. The significant data collected were considered to be an innovative breakthrough in marine mammal science, where a single animal can be used to demonstrate the capability that may exist within a species. The use of behavioral, psychometric techniques became the standard to investigate hearing thresholds of captive odontocetes. Since this experiment, behavioral audiograms measuring the auditory sensitivity have been collected for the harbor porpoise, *Phocoena phocoena* (Andersen, 1970; Kastelein et al., 2002), killer whale, *Orcinus orca* (Hall and Johnson, 1972), Amazon River dolphin, *Inia geoffrensis* (Jacobs and Hall, 1972), beluga whale, *Delphinapterus leucas* (White et al., 1978), Eastern Pacific bottlenose dolphin, *Tursiops spp.* (Ljungblad et al., 1982), false killer whale, *P. crassidens* (Thomas et al., 1988), Chinese River dolphin, *Lipotes vexillifer* (Wang et al., 1992), Risso’s dolphin, *Grampus griseus* (Nachigall et al., 1995), tucuxi, *Sotalia fluviatilis guianensis* (Sauerland and Dehnhardt, 1998), and the striped dolphin, *Stenella coeruleoalba* (Kastelein et al., 2003).

For more than 30 years, psychometric research with marine mammals developed based on the success using a single or few research subjects. Considerable hearing threshold differences between individual animals of different ages and sexes have been demonstrated in previous behavioral hearing research (Ridgway and Carder, 1997), differences that may not be evident when only a single animal subject is used. Such psychometric analysis was constrained by the significant investment to train the subjects for the behavioral paradigm and to maintain the animals in captive environments to conduct thorough research.

An alternative to the psychometric methods of measuring behavioral audiograms was the collection of auditory evoked potentials (AEP). When an acoustic stimulus is presented, the cells within the auditory pathway are excited. When this occurs, AEPs are the far-field electrophysiological recording of the resulting small voltages generated by the brain’s neural activity. Measuring the evoked responses to stimuli became a valuable and advantageous method to collect auditory data, requiring minimal training and reduced time. The characteristics of AEPs were first described using invasive, intracranial electrodes in the dolphin brainstem (Bullock et al., 1968) and in the cerebral cortex (Popov et al., 1986), and using noninvasive extracranial electrodes (Ridgway et al., 1981). In more recent times, noninvasive techniques have been employed to investigate auditory brainstem responses (ABR), a type of AEP involving a series of five to seven “waves” evoked by clicks or short tone bursts of acoustic stimuli. ABRs to tone pips were successfully measured...
used to measure hearing thresholds and collect audiograms in dolphins (Popov and Supin, 1990a, 1990c).

Measuring hearing thresholds was more precise when using envelope-following responses (EFR), the occurrence in which ABRs follow the envelope of a sinusoidally amplitude-modulated (SAM) tone burst (Dolphin et al., 1995; Supin and Popov, 1995). The advantages of using SAM stimuli instead of brief tone pips were: (1) the intensity of a long tone burst was characterized by its rms sound pressure, which could provide a basis for correct comparison of behavioral and AEP data, and (2) EFRs contained many evoked potential cycles instead of one, increasing the precision of the response detection, in particular, by the use of Fourier analysis.


Although measuring AEPs has proven to be a reliable technique for investigating the hearing sensitivity of odontocetes, it remained unresolved how the electrophysiological thresholds compared with the thresholds collected by behavioral techniques. Some analyses attempted to compare data gathered using the two different methods (Szymanski et al., 1999). In that study, two killer whales were used to measure both ABR and behavioral audiograms. The stimuli used for the electrophysiological measurements were cosine-gated tone bursts, and ABR thresholds were determined by calculating the minimum amount of stimulus power needed to generate an adequate ABR response. EFRs were not used in that experiment, and the frequency ambiguity of ABR thresholds was described. The behavioral methods varied slightly to maintain the motivation of the animal subjects, and the final thresholds were the average of four reversals.

In the current study, the hearing capability of a false killer whale was measured using both behavioral and AEP techniques, using the same animal subject as well as the same experimental and acoustical conditions. The objective of this project was to investigate and compare auditory thresholds collected by psychometric and AEP techniques, more specifically, EFR procedures. Based on the paradigm differences and the conservative nature of the animal subject, it was expected that the behavioral methods would produce thresholds lower than the ABR thresholds.

II. METHODS

A. Animal subject

Both the behavioral and AEP data were collected from a single animal subject named Kina, an approximately 30 year old, female false killer whale (*Pseudorca crassidens*). Kina was about 3.7 m in length and weighed 487 kg. The animal was housed in floating pens at the HI Institute of Marine Biology on Oahu, HI, and had been the subject of previous hearing and echolocation research, including a masked hearing study (Thomas et al., 1990), the measurement of echolocation transmission beam patterns (Au et al., 1995) and experiments collecting ABRs during echolocation (Supin et al., 2003; Supin et al., 2004).

B. Electronic equipment

The pure-tone stimuli used during the behavioral measurements were created with a Wavetek FG3B Sweep Function Generator. The frequencies tested were 4, 5, 7, 8, 10, 14, 16, 19, 20, 22.5, 27, 32, 38, and 45 kHz. The input voltage of the stimulus was 3 V peak-to-peak. The stimulus was sent to a custom-built signal shaping box that could attenuate the tone in 1-dB decrements, control the trial sequence and trial condition, and turn the signal on and off with a 20-ms rise and fall time. The signal was then projected through an ITC 1042 60 mm spherical piezoelectric transducer. A Technox TDS 1002 Oscilloscope was used to monitor the signal sent from the signal-shaping box to the transducer. A Biomax 8235 hydrophone that had a flat frequency response (+3 dB) up to 200 kHz was used to calibrate the frequency levels of the signal as it was received in the center of the hoop where the animal would be positioned during the stimulus presentation. The calibrations were conducted before the data were collected and not during the trials.

In AEP experiments, SAM tone bursts were digitally synthesized with a customized LABVIEW 6 data acquisition program from a desktop computer implemented with a National Instruments PCI-MIO-16E-1 DAQ card, using an update rate of 200 kHz. The SAM tone bursts were 20 ms long, with a modulation rate of 875 Hz, a modulation depth of 100%, and a variable carrier frequency. The stimulus was sent from the DAQ card to the same amplification, attenuation, monitoring, and projection equipment as in the behavioral experiments.

The AEPs were received through 10-mm gold disc electrodes that were mounted in rubber suction cups and placed on the animal’s skin along the dorsal midline, with the active electrode about 5 cm behind the blowhole and the reference electrode on the animal’s back, anterior to the dorsal fin. These responses were amplified by 10,000 with an Iso-Dam Isolated Biological Amplifier, bandpass filtered (for anti-aliasing protection) with a Krohn-Hite Model 3103 filter (bandpass of 200–3000 Hz), and transferred to an analog input of the PCI-MIO-16E-1 DAQ card. To extract the recorded AEP from noise, the signal was digitized at a rate of 16 kHz, and 1000 samples were averaged to stimuli presented at a rate of 20/s, thereby extending the entire trial to about 1 min.

C. Experimental setup

The experimental conditions were nearly the same for both the behavioral and the AEP experiments. Both kinds of experiments were conducted within the same test pen, a 6 × 9 m floating pen in Kaneohe Bay, off the island of Oahu, HI (Fig. 1). This wire-fence enclosed pen was supported by floating buoys under the pen’s wooden frame. The transmitting hydrophone was suspended 1 m below the water surface and secured at one corner of the pen deck. An acoustic baffle
was made of a 0.6 × 0.9 m aluminum sheet that was covered with neoprene on the side facing the transducer, and was hung at the surface of the water at the half-distance between the transducer and the animal (Fig. 2). The water’s surface will reflect additional sound underwater, and the baffle was used to reduce and block some of this surface reflection that could have reached the animal. The hoop was placed 2 m from the sound source and fixed firmly from a wooden beam that stretched across the pen deck. A Styrofoam ball response paddle was attached to the wooden beam directly above the hoop and the surface of the water. During the intertrial intervals, the animal was trained to station on a Styrofoam float at the water surface about 3 m away from the transducer, and about 5 m away from the hoop. A small transmitting hydrophone was placed in the water near this float and projected only a 7-kHz tone. This tone was used as a signal to send the animal to the hoop at the beginning of each trial.

D. Behavioral audiogram measurements

The behavioral audiogram of the false killer whale was measured by using a go/no-go modified staircase procedure (Schusterman, 1980) in 2001 and 2004. The 7-kHz tone was played to send the whale to an underwater hoop. When the whale was positioned correctly with her pec fins touching the hoop, a 3-s, pure-tone test stimulus was transmitted underwater from the spherical transducer. If the whale heard this sound, she exited the hoop and touched a response paddle that was located directly above the hoop and the surface of the water. This correct “go” response was followed by the trainer’s whistle and fish reinforcement. For a correct rejection or a “no-go” response, no sound was projected, and the whale was required to remain in the hoop for the full 10-s trial, after which the trainer whistled to signal to the whale that she had performed the correct response. The whale was again rewarded with fish. For an incorrect detection or a “false alarm,” the whale would indicate that she heard a sound and touch the paddle. However, no sound was played and therefore, no reward or whistle was given. The whale returned to her stationing float, and the next trial proceeded. When an incorrect rejection, or “miss” occurred, the whale remained in the hoop and did not respond to the sound that was played. The trainer would call the animal back to her stationing float with no reward at the end of the 10-s trial.
Ten warm-up trials were presented before each session to gauge the whale’s response behavior with five of the trials with a sound transmitted and five without. The sounds played in the warm-up trials were at intensity levels that were at least 20 dB above the presumed threshold based on published audiograms, loud enough for the whale to detect without difficulties. The session only proceeded if 80% of the warm-up trials were correct. The trials following warm-ups were conducted in blocks of ten, with 50% of each block containing a signal present and the other 50% with a signal absent. The sequence of the trials was randomized based on a modified Gellermann series (Gellermann, 1933), with no more than three consecutive trials of the signal present or absent to avoid any bias of the whale to respond the same way to every trial.

Every correct go-response was followed by a stimulus intensity reduction of 2 dB, until the animal made a miss response, after which the intensity was increased by 2 dB. This change in direction of intensity level was defined as a reversal. The intensity continued to be increased until there was another correct go-response, which was followed by an intensity decrease, another change of direction or reversal.

For each session, one frequency threshold was tested, and the value of that threshold was the average intensity level of five reversals. It was determined from a preliminary study that there was no significant difference between a threshold calculated from five reversals versus a threshold calculated from ten reversals. Thresholds were equivalent and completed within a shorter amount of time if only five reversals were used. The final threshold for each frequency was calculated when two consecutive sessions contained reversal thresholds within 3 dB. Finally, this entire staircase procedure was repeated to determine the thresholds at 4, 5, 7, 8, 10, 14, 15, 16, 19, 20, 21, 22.5, 24, 27, 32, 38, and 45 kHz. The animal’s hearing sensitivity was tested at 64 kHz, and demonstrated that she was not able to hear this high frequency when it was played at the highest intensity level possible for the equipment available, which was 132 dB. As a result, no further testing was conducted at frequencies above 45 kHz.

E. AEP audiogram measurements

There were three main differences in the experimental procedure between the AEP and behavioral audiograms. One was that gold disc electrodes in rubber suction cups were placed on the animal. The second difference was that no behavioral reporting response was required from the animal. The animal remained in the hoop for a trial length of about 1 min while the stimulus was projected underwater and the AEP was recorded. The final difference was the stimulus type that was presented, in this case being a 20-ms SAM tone-burst repeated 1000 times, as opposed to the 3-s pure-tones generated for the behavioral audiogram.

Following the application of the suction cups, the animal was sent to the underwater hoop from a stationing float by a 7-kHz tone. When the animal was positioned correctly with her pectoral fins touching the hoop, the SAM tone bursts were projected through the transducer. After about 1 min, the trainer whistled to indicate the end of the trial, and the animal was rewarded with fish. The amplitude of the signal was reduced in 5-dB steps, until the EFR response recorded on the computer could no longer be distinguished from the background noise. An average of five stimulus intensities were presented at each frequency, and an average of three frequencies was tested in 1-h sessions. Sessions were conducted twice a day. The frequencies tested were in quarter-octave steps: 4, 4.7, 5.6, 6.7, 8, 9.5, 11.2, 13.5, 16, 19, 22.5, 27, 32, 38, and 45 kHz. A total of three AEP audiograms were measured, one in May 2001, a second in August 2001, and a third in April 2004. An average of two weeks of consecutive days was needed to complete each of these audiograms.

No additional training was required for the whale when the task was switched from behavioral to AEP experiments. The very rapid transfer between paradigms was most likely due to the differences in the experimental setup, the most obvious of which included the suction cups attached to the animal and the SAM tone-bursts. It is possible that these distinct features allowed for the whale to easily discriminate between the two experimental tasks.

III. RESULTS

A. Behavioral audiograms

The first behavioral audiogram from 2001 was a preliminary study that included only a partial audiogram with thresholds for only five frequencies. The behavioral audiogram from 2004 was much more complete, with 16 frequencies covering at least 4 octaves (Fig 3). The complete audiogram from 2004 had the U-shape curve characteristic of mammalian hearing. The thresholds were very similar over the 3-yr gap, with some values differing by as little at 2.6 dB at 32 kHz, and as great as 12.1 dB at 38 kHz (Table I). However, the shapes of the two curves were very consistent, with comparable regions of best sensitivity and steep high frequency range. The region of best sensitivity was from 16 to 24 kHz, with the lowest threshold of 69 dB at 20 kHz. Above 24 kHz, the thresholds increased significantly at a rate of about 28 dB per octave, with a high frequency cutoff.
at 45 kHz. The low frequency thresholds decreased as the frequencies increased, at a rate of about 5–17 dB per octave.

A total of 16 frequencies were tested for the 2004 audiogram, 11 of which required only two consecutive sessions that resulted with reversal averages within 3 dB. Three frequencies needed three sessions to meet this requirement, one frequency needed four sessions, and one frequency needed five sessions. There was a very low false alarm rate throughout the experiment. Over 76% of the sessions consisted of zero false alarms, and of the remaining sessions with false alarms, the rate was about 5%.

B. AEP audiograms

The electrophysiological responses from the AEP collection is presented in Fig. 4 and depicts how EFRs recorded at different stimulus intensities decreased from 125 to 90 dB by 5-dB steps. As the intensity decreased, the EFR amplitude synchronously decreased until the response disappeared in noise. For a better evaluation of the response amplitude, these wave forms were Fourier transformed to obtain their frequency spectra [Fig. 4(b)]. The spectra contained a definite peak at the stimulus-modulation frequency of 875 Hz. The magnitude of this peak was taken as a measure of the response magnitude. It was plotted as a function of stimulus intensity (Fig. 5) and approximated by a regression line. The crossing point of this line with the zero-magnitude level was calculated as the threshold estimate for that frequency.

The three AEP audiograms were consistent over time (Fig. 6). All three had a range of best hearing sensitivity from 16 to 22.5 kHz with the lowest threshold of 80.9 dB at 22.5 kHz (Table II). The thresholds at lower frequencies all had a slope that declined gradually as frequency increased, about 8–18 dB per octave. The thresholds at higher frequencies sharply increased, about 32 dB per octave, and quickly reached higher intensity at 45 kHz. All three AEP audiograms had relatively consistent threshold values at the lower frequencies, some with differences less than 1 dB. The May 2001 and April 2004 thresholds for the higher frequencies were closer, with some thresholds fluctuating by as little as 0.2 dB. However, the August 2001 AEP audiogram had the highest thresholds for the higher frequencies that were noticeably different, despite the consistency of the lower frequencies with the other two AEP audiograms.

IV. DISCUSSION

A. Comparison of behavioral and AEP audiograms

The results of the behavioral audiograms demonstrated that the data accumulated from psychometric methods were

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<th>Freq (kHz)</th>
<th>Threshold 2001 (dB re: 1 μPa)</th>
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<th>Difference (dB re: 1 μPa)</th>
<th>Average (dB re: 1 μPa)</th>
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FIG. 4. Examples of (a) EFR wave forms and (b) their frequency spectra for a threshold determination at 27 kHz.

FIG. 5. EFR magnitude dependence on stimulus intensity (by the records presented in Fig. 2).
TABLE II. Auditory thresholds and average value of three AEP audiograms of a false killer whale. Also included are the averaged values of all three auditory thresholds for each frequency. Units are in dB re: 1 μPa.

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<th>Freq (kHz)</th>
<th>Threshold May 01 (dB re: 1 μPa)</th>
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<th>Threshold April 04 (dB re: 1 μPa)</th>
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FIG. 6. Three AEP false killer whale audiograms measured over three years. Also included is a curve of the average value of all three audiograms.

A similar gap between AEP and behavioral threshold estimates was found in two killer whales *O. orca* (Szymanski et al., 1999), with an average of 12 dB more sensitive behavioral thresholds. However, there were some technical differences between that and the present study. The tone burst stimuli used were not SAM tone bursts, and ABRs not EFRs were measured. As mentioned earlier, the advantages of using SAM stimuli instead of brief tone pips were that the intensity of the longer tone burst could be characterized by its rms sound pressure, thereby providing a basis for correct comparison of behavioral and AEP data, and that the many evoked potential cycles contained within an EFR increased the response detection precision and analysis. Despite these discrepancies, the final conclusions in the 1999 study were somewhat similar to those of the present study, that both the behavioral and ABR audiograms had consistent shapes with higher ABR threshold levels.

The significant feature of the different stimulus types clearly distinguished each technique and possibly contributed to the data differences. SAM tone bursts of 875-Hz modulation rate and 20 ms long were necessary to collect AEPs. Contrary to this, behavioral methods involved pure-tone signals with a duration of about 3 s. The longer pure-tone may have been more detectable and unambiguous, and consequently allowed for a clearer response by the subject. For EFR responses, temporal summation was limited by SAM cycle duration which can be as short as 1.14 ms. As shown by Johnson (1968), hearing thresholds decreased as stimulus duration increased from a fraction of milliseconds to hundreds of milliseconds. Thus, the threshold difference between behavioral measurements and AEP measurements reflected the real temporal summation processes in the auditory system rather than an imprecision of one or another method.

It is important to remember that any estimate of a threshold, AEP or behavioral, was arbitrary, and that the results depended on the used threshold criterion. However, the good correspondence of behavioral and AEP thresholds, with different summation times taken into account and with the...
use of the above-described criteria, indicated that both methods provided good estimates of the real sensitivity of the auditory system.

One of the practical differences between the psycho-physical and AEP techniques was the shorter amount of time required to complete the AEP audiogram. For the behavioral audiogram, each session lasted approximately 30 min, and a total of 42 sessions were needed to test all of the frequencies. The time invested for data collection in this portion was about 2 months with a well trained and experienced animal subject. This amount of time does not include the years of behavioral training that was initially dedicated and required for this subject.

One session for an AEP audiogram lasted about 45 min, during which three frequencies could be tested. Therefore, one AEP session tested three frequencies in about the same amount of time that one behavioral session tested one frequency. When these sessions were conducted twice a day, the total time dedicated to this phase was an average of two weeks, remarkably more condensed and still with results comparably robust to the behavioral paradigm. With such a reduced time and training requirement, and the minimum participation required by the animal subject, the AEP technique for measuring auditory thresholds appeared more suitable and convenient to a wider number of research opportunities that include untrained or stranded animals.

ABR audiograms served as a resource for evaluating the sensitivity and functionality of auditory systems for many vertebrate species, including birds (Brittan-Powell et al., 2002), bats (Wenstrup, 1984), fish (Kenyon et al., 1998), and humans (van der Drift et al., 1987; Mitchell et al., 1989; Watson, 1996). When the data from the AEP audiograms were compared to behavioral audiograms, most AEP thresholds were higher than behavioral thresholds, although the differences were usually not significant. There was a common conclusion that the advantages of the electrophysiological technique included the rapid evaluations and reliability of data, allowing for good predictions of basic audiogram shape.

B. Manifestation of hearing loss

When the results from our study were compared with the behavioral audiogram of another false killer whale (Thomas et al., 1988), the thresholds diverged considerably, with two distinguishing features. The first was that the animal subject from the 1988 study heard frequencies as high as 115 kHz reasonably well, and at about the same SPL that Kina heard 45 kHz. The high frequency cutoff for the current audiogram was about 70 kHz lower than the previous audiogram. The second feature was the different frequency region of best sensitivity and the corresponding sensitivity thresholds. In this 2004 study, the region was between 16 and 24 kHz with the lowest threshold at 69 dB at 20 kHz. However, in the 1988 study, the region of best sensitivity was between 32 and 64 kHz, and with thresholds as low as 39 dB at 64 kHz. Therefore, the current audiogram had a lower high frequency cutoff, in addition to the region of best sensitivity shifted to lower frequencies and heard only at higher threshold levels, clearly indicating that the whale hearing sensitivity lessened when compared to the subject of the previous study. Not only could the whale not hear higher frequencies, but also her best hearing was at lower frequencies and higher amplitudes.

Both audiograms were made using standard psychometric techniques and pure-tone stimuli. Despite similar paradigms, such discrepancies could have resulted from the different locations of each experiment, where the earlier subject lived in a concrete and virtually quiet tank, and the current subject resided in the open waters of Kaneohe Bay where the ambient noise level has been documented, in particular the noise produced snapping shrimp (Au and Banks, 1998). This noise has an extremely broad frequency spectrum with energy beyond 200 kHz with a peak frequency at about 2 kHz. It does not follow from those data that the ambient noise in Kaneohe Bay may specifically mask sounds above 45 kHz.

In 1990, the same false killer whale as the present study was also the experimental subject for a masked hearing study also situated in Kaneohe Bay, HI (Thomas et al., 1990). That experiment was conducted 14 years prior to the current study, and at that time, the whale’s masked hearing audiogram depicted exceptional hearing capabilities, indicating good hearing sensitivity. Therefore, it has been hypothesized that this animal probably suffered some hearing loss associated with age or presbycusis, an occurrence demonstrated among older bottlenose dolphins (Ridgway and Carder, 1997; Ketten et al., 2001) and a beluga whale (Finneran et al., 2003).

C. Perspectives for future studies

Auditory threshold information exists for only about 12% of odontocete species, creating a significant deficiency of scientific evidence for the potential hearing damage and behavioral impacts of anthropogenic noise on marine mammals. There is a serious need for additional research on more cetacean species, especially since the accessibility of marine mammals for behavioral research is rare. Research opportunities are limited because there are very few laboratory facilities in the world where marine mammals are available for scientific research. The behavioral and electrophysiological techniques to investigate hearing are conducted very differently, one with considerable time and effort involved in training psychometric methods, versus the other with very limited subject participation required to collect AEPs, an advantage that may strengthen the need for AEP techniques by broadening its applicability to untrained animal subjects. There are differences with signal type used in the behavioral and AEP techniques that may influence the research outcomes. However, with these differences taken correctly into account, both techniques give comparable results.

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