Measuring hearing in the harbor seal (*Phoca vitulina*): Comparison of behavioral and auditory brainstem response techniques

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(Received 8 January 2001; revised 30 September 2002; accepted 10 October 2002)

Auditory brainstem response (ABR) and standard behavioral methods were compared by measuring in-air audiograms for an adult female harbor seal (*Phoca vitulina*). Behavioral audiograms were obtained using two techniques: the method of constant stimuli and the staircase method. Sensitivity was tested from 0.250 to 30 kHz. The seal showed good sensitivity from 6 to 12 kHz [best sensitivity 8.1 dB (*re* 20 $\mu Pa^2 \cdot s$) RMS at 8 kHz]. The staircase method yielded thresholds that were lower by 10 dB on average than the method of constant stimuli. ABRs were recorded at 2, 4, 8, 16, and 22 kHz and showed a similar best range (8–16 kHz). ABR thresholds averaged 5.7 dB higher than behavioral thresholds at 2, 4, and 8 kHz. ABRs were at least 7 dB lower at 16 kHz, and approximately 3 dB higher at 22 kHz. The better sensitivity of ABRs at higher frequencies could have reflected differences in the seal's behavior during ABR testing and/or bandwidth characteristics of test stimuli. These results agree with comparisons of ABR and behavioral methods performed in other recent studies and indicate that ABR methods represent a good alternative for estimating hearing range and sensitivity in pinnipeds, particularly when time is a critical factor and animals are untrained. [DOI: 10.1121/1.1527961]

PACS numbers: 43.80.Lb, 43.80.Nd [WA]

I. INTRODUCTION

Audiometric testing of pinnipeds usually has been conducted using standard psychoacoustic methods such as the method of constant stimuli or the staircase method (Gelfand, 1998). These methods have been used to determine hearing sensitivities in the California sea lion (Schusterman *et al.*, 1972; Schusterman, 1974), harbor seal (Mohl, 1968; Turnbull and Terhune, 1990; Terhune, 1991; Kastak and Schusterman, 1998), elephant seal (Kastak and Schusterman, 1998), harp seal (Terhune and Ronald, 1971, 1972), northern fur seal (Moore and Schusterman, 1987; Babushina *et al.*, 1991), and ringed seal (Terhune and Ronald, 1975). To date, however, only a few individuals of each species have been tested. The paucity of audiograms for most pinniped species makes it difficult to establish normative hearing capabilities and intra- and interspecies variation. Such information is needed to interpret measures of hearing loss and to estimate risk factors for populations of animals. To accomplish this, hearing measurement techniques that are conducive to testing multiple untrained animals must be refined.

The auditory brainstem response (ABR) method may be used as an alternative for determining hearing sensitivity across a range of frequencies. The ABR directly measures whole-brain evoked potentials produced by neurophysiological activity as it travels from the auditory nerve to the brain. The ABR has been used to examine hearing sensitivities in a variety of species, including harbor seals and California sea lions (Bullock *et al.*, 1971), bottlenose dolphins (Supin *et al.*, 1993; Ridgway *et al.*, 1981), and killer whales (Symanski *et al.*, 1999).

While both behavioral and ABR methods are useful for determining hearing ranges and sensitivities, each has limitations. Behavioral methods are preferred because they produce the most sensitive threshold measurements (Fay, 1988). Unfortunately, the animal training required prohibits testing of free-ranging animals or large numbers of individuals. For example, only four harbor seals have been tested since work began on this species in 1968. The ABR presents an attractive alternative method to behavioral testing because it requires less time to complete and no animal training. However, it usually yields higher thresholds (Katz, 1994) and is less reliable at the extremes of an animal's hearing range, particularly at low frequencies (Erhet, 1983). ABRs are generally thought to be 10–15 dB less sensitive than behavioral methods (Gorga *et al.*, 1988), but careful work with humans has shown that an experienced investigator can obtain agreement within 5–10 dB (Sininger, 1993).

A comparison of behavioral and ABR measurements is most useful when the two methods are performed on the same individual, thus controlling for individual differences in hearing sensitivity. One recent study measured audiograms of two individual killer whales (Symanski *et al.*, 1999) using both methods and found good agreement (±5 dB), suggesting that the ABR can provide a good suprathreshold estimate of hearing range and sensitivity in toothed whales (Ridgway *et al.*, 1981; Popov and Supin, 1990; Dolphin, 1995; Symanski *et al.*, 1995).

The present study arose from a project investigating the effects of low-frequency impulse noise on hearing sensitivity in pinnipeds (Bowles *et al.*, 1998). The objective was to as-

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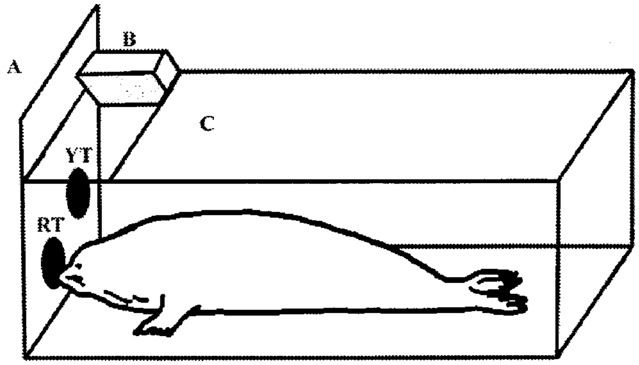

FIG. 1. Sound isolation box illustrating targeting stations and locations of speakers. A—Location of trainer during trials, B—speaker, C—location of speaker mounted to the roof of the box, RT—ready target, and YT—yes target.

sess the potential for temporary threshold shifts (TTS) as a result of exposure to simulated sonic booms in rehabilitated pinnipeds of three species [California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*), and elephant seals (*Mirounga angustirostris*)]. Because behavioral methods are considered the "gold standard" for audiometric testing (Fay, 1988), it was necessary first to establish that ABR and behavioral methods yielded comparable results.

II. MATERIAL AND METHODS

A. Subject

The subject was an adult female harbor seal (NMFS No. SWCPV9614B). She was a beached, rehabilitated animal residing at the Wild Arctic facility at SeaWorld, San Diego. The seal was 4 years old at the time of testing and was naïve to testing procedures when the study began.

B. Behavioral methods

1. Experimental conditions

Behavioral testing was conducted between August 1998 and September 1999, following six months of behavioral training to the experimental procedures. All testing was completed in an acoustic attenuation box that reduced ambient noise by 10–20 dB from 200 Hz to 30 kHz, increasing in attenuation with increasing frequency. The attenuation box measured $1.78\times0.76\times0.76$ m^3 and was constructed from 1.3-cm-thick plywood and 5.25-cm-thick Sonex acoustic foam (Fig. 1). Two Polk M4 speakers were used in parallel to generate sound stimuli. One speaker was mounted above and to the side of the seal's head during testing. The speaker was approximately 1 m away and at a 45-degree angle to the animal's ear. A second speaker was mounted on the roof of the box 1.2 m behind the seal's head to reduce reflections, thereby increasing the area around the head with an acceptable variance of signal strength ($<\pm2$ dB).

Sound levels at each test frequency were calibrated before and after each trial block using ACO 7012 and ACO 7013 microphones and a Spectral Dynamics 780 Signal Analyzer. The microphones themselves were calibrated daily with a Bruel & Kjaer 4228 pistonphone as well as a Bruel & Kjaer 4230 acoustic calibrator. The calibration before each test block was done with a "dummy" seal head in place at the appropriate location, and a microphone at the approximate location of the seal's meatus.

The seal was tested using a go/no-go procedure. Two targeting stations were placed at one end of the testing box (Fig. 1). The seal was trained to enter the box on command and station on the "ready" target (RT). Each trial began with the trainer giving an auditory command ("target!") that cued the seal to position herself at the RT. The seal nearly always remained motionless with eyes closed while at the RT. Closure of the eyes was a convenient idiosyncratic behavior for this animal—it reduced the chance that she could detect visual cues from the trainer. Latencies to tone onset after the seal had positioned herself on the RT were varied from 3 to 8 s. When the subject heard a tone she touched the "yes" target (YT), then returned to the RT. The seal had to move to the YT within 2 s after the tone was played in order to receive a positive response score (hit). A false alarm was scored if the seal moved from the RT to the YT at any other time during a trial session. If the seal touched the YT more than 2 s after the tone, or not at all, the response was scored as a negative response (miss) and the trainer gave an auditory cue to signal the end of the individual trial. A false response was scored if the seal touched the YT during a catch trial—a trial performed with no stimulus to determine the rate of spontaneous reactions.

Two to five testing blocks were conducted per day. Each testing block consisted of 26 trials, with tones presented 70% of the time, and catch trials 30%. Following each testing block, the subject was allowed access to a holding pool. The seal appeared strongly motivated to perform the experiments, spontaneously entering the test box whenever the opportunity was presented. If either the false response rate, false alarm rate, or any combination of the two rose above 10% in any given testing block, that testing block was terminated and the animal was given a rest period before testing resumed.

2. Method of constant stimuli (MCS)

Thirty percent of all behavioral trials (1111 of 3699 trials) were conducted using the method of constant stimuli. Frequencies tested were 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 kHz. Tones (500-ms duration, 0.5-ms rise-time, Blackman-filtered) were delivered using a laptop computer and the Wave SE (Turtle Beach) software program. Stimulus amplitudes were calibrated before each testing session. A testing block consisted of one stimulus frequency presented 18 times at various amplitudes interspersed with blanks (30% catch trial rate). The presentation order of stimuli was randomized by performing three iterations of the MATLAB (The MathWorks, Inc.) "randomize" function. The minimum difference between any two stimulus amplitudes was set at 5 dB to control for variability in signal strength. Each stimulus frequency was tested in at least four testing blocks for a total of approximately 80 trials per frequency. The seal was reinforced verbally and with a food reward for responding correctly to both tones (moving to the YT) and catch trials (remaining on the RT). False responses were not reinforced.

3. Staircase method (SM)

Seventy percent of all behavioral trials (2588 of 3699 trials) were completed using an adaptive up–down procedure (Levitt, 1970) carried through at least five reversals, referred to herein as the staircase method. Tone amplitudes were decreased in 5-dB increments until the seal's response was scored as a miss. Following a miss, the next tone was increased by 10 dB. If the seal scored a hit on this tone, the intensity was lowered in 5-dB increments until another miss was scored. Each series of descending intensities was termed a "descent." Five descents were performed in each trial block, resulting in five threshold measurements per block. This approach was used to minimize the number of trials needed to measure the seal's thresholds and to ensure that the relatively inexperienced seal was exposed to as few inaudible stimuli as possible.

Frequencies tested were 0.25, 0.50, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 22.0, 25.0, and 30.0 kHz. Tones were delivered using a Pragmatic 2411A Arbitrary Waveform Generator (ARB) and a Yamaha P1150C amplifier. Each testing block consisted of 18 tone presentations at a fixed frequency and eight randomly placed catch trials (30% catch trial rate). The seal was reinforced only after correctly responding to tone trials. This change in reinforcement schedule from the MCS method was desirable because it reduced the number of trial blocks that had to be eliminated due to a high false response rate (see Sec. IV).

C. Determination of behavioral thresholds

For the MCS trials, the percentage of positive responses was calculated for each decibel level presented during that day's trial session. The lowest level at which the animal responded positively 70% of the time was determined to be the threshold. This percentage approximated the location on the psychometric function that would be produced by the staircase method (Gelfand, 1998; Nachtigall *et al.*, 2000). Trial blocks in which the false response rate, false alarm rate, or both rose above 10% were excluded from the determination of thresholds.

During SM trials, thresholds were determined by noting the lowest intensity in each descent that scored a hit. The midpoint between this intensity and the intensity at which the animal scored a miss during that descent was considered the threshold. This method yielded a threshold criterion of approximately 71% (Nachtigall *et al.*, 2000). Five thresholds per trial block were generated, and the mean of these measurements was taken as the threshold for the block.

For both methods, the sensitivity (d') of the testing paradigms was calculated using methods given in Elliott (1964), Swets (1964), and Gelfand (1998). Sensitivity was used as a measure of the separation between the seal's criterion level for false responses to noise alone and correct responses to the noise-signal combination. It was calculated as the difference in mean response level to the noise-signal combination versus noise over the standard deviation of all levels.

D. ABR experimental conditions

Evoked potential measurements were completed at the Wild Arctic facility on 30 August 1999. During testing the seal was placed on a restraint board fitted with 2-in nylon straps and a neck board in order to minimize movements and the range of head motion, and was sedated with diazepam (0.15 mg/kg) to reduce muscle activity. This dosage is not believed to affect ABR morphology or amplitude (Doring and Daub, 1980). Several other physiological parameters were measured in addition to ABRs. Electrocardiogram (ECG) electrodes were placed on opposite sides of the spine, just posterior to the flippers, electrooculogram (EOG) and electromyogram (EMG) electrodes were placed into the muscle just above the eye, and a respiration band was fitted around the rib cage.

E. ABR measurement

ABRs were measured using a Bio-Logic Traveler SE computer running the Evoked Potential (EP) program. This is a turnkey measurement system that can generate stimulus waveforms and simultaneously acquire evoked responses. Three platinum-iridium electrodes were inserted subdermally on the seal's head. The reference electrode was placed between the right auditory meatus and mastoid, the active electrode was placed at the vertex of the head along the plane of the reference electrode, and a ground was placed in the nape of the neck. Impedances of electrodes were kept under 10 kΩ and the differences between any two electrodes were kept at 3 kΩ or less.

F. ABR stimuli and presentation

Wideband stimuli (clicks) as well as tone bursts (2, 4, 8, 16, and 22 kHz) were presented. Stimuli were generated using the ARB. The Bio-Logic system collected all ABR responses. Each stimulus was five cycles in length, with two cycles each for the rise and decay, and one cycle at the plateau (Hall, 1992). The rise and decay were Blackman-filtered. Clicks and tone pips were delivered at a rate of 29.3/s. This rate did not affect ABR waveform morphology or amplitude, and allowed rapid testing at each stimulus amplitude. All waveforms in this experiment were presented through a Polk M-4 Studio Tweeter. Levels were calibrated with two ACO 7013 microphones, one of which was placed at the approximate location of the seal's meatus and oriented towards the speaker as the meatus would be during trials ("ear microphone"). The other microphone was placed approximately 30 cm from the tweeter and 70 cm from the animal's head, and was used to verify stimulus levels during trials ("reference microphone"). Sound levels presented during the experiment were calibrated for the ear microphone position and the corresponding reference microphone sound level was noted. At the conclusion of the experiment, spectra were recorded for all waveforms presented, both at high amplitudes and at near-threshold levels.

TABLE I. Mean hearing thresholds using method of constant stimuli.

Frequency (kHz)	Threshold (dB *re* 20 $\mu Pa^2 \cdot s$) RMS	Standard deviation (dB *re* 20 $\mu Pa^2 \cdot s$) RMS	Threshold (dB SPL *re* 20 μPa)	Total no. of trials
0.25	61.0	4.2	64.0	97
0.50	51.8	4.5	54.8	67
1.0	45.8	4.8	48.8	69
2.0	42.0	7.4	45.0	107
4.0	31.5	5.0	34.5	207
6.0	23.1	4.9	26.1	150
8.0	22.9	7.4	25.9	126
10.0	20.8	6.3	23.8	162
12.0	14.7	4.0	17.7	51
14.0	20.2	5.4	23.2	75

G. Determination of ABR thresholds

Latency and amplitude of the most prominent ABR waveform peaks were used to quantify responses. Response waveforms were considered valid only if their electrical variability was within a pre-set range (1.2 μV). Signals with excessive myogenic noise or otherwise outside of the pre-set range were discarded. Approximately 1000 valid response waveforms were collected and averaged at each stimulus level. For each frequency, stimulus level was dropped in 10-dB increments until the most prominent peak was reduced in amplitude. At this point the stimulus level was decreased in 5-dB increments until the peak could no longer be detected. Two to five replicates were collected at each stimulus level for each frequency. Thresholds were determined *post-hoc* by noting the lowest stimulus level where the most prominent peak was detectable, repeatable in replicates, and above the background noise of zero-amplitude ABR responses ($\pm$0.3 μV).

H. Comparison of stimulus levels

In order to directly compare audiograms using auditory stimuli of different durations, a normalizing procedure was followed to equate stimulus duration. Time waveforms for each stimulus were recorded and the RMS (root-mean squared) sound pressure (RMS SPL in Pascals) for each stimulus intensity was calculated. Sound pressure was converted to decibels (*re* 20 μPa). The duration of the stimulus was then used to calculate energy level in dB *re* 20 $\mu Pa^2 \cdot s$. In the following sections, the unit dB will always be referenced to 20 $\mu Pa^2 \cdot s$.

III. RESULTS

A. Behavioral data

1. Method of constant stimuli (MCS)

Hearing threshold estimates for tones between 0.25 and 14 kHz were generated using the method of constant stimuli (Table I). The seal displayed best sensitivity at 12 kHz (14.7 dB), with the best range ($\pm$10 dB) between 6 and 14 kHz. Thirteen of 79 testing blocks (16%) had false alarm rates above 10% and were therefore not included in the analysis. False response rates were <9% during catch trials.

2. Staircase method (SM)

The best sensitivity measured using the staircase method was 8.1 dB at 8 kHz, and best range was 6–12 kHz (Table II). The seal displayed a notch in sensitivity centered at 2 kHz (231 trials, 52 reversals); thresholds at 1 and 3 kHz were approximately 12 dB lower. Interestingly, a similar increase in threshold near the same frequency (1.6 kHz) was observed in an earlier study with a harbor seal (Kastak and Schusterman, 1998).

The seal performed more consistently during SM trials. False response rates were <6% during catch trials. Fewer

TABLE II. Mean hearing thresholds using descending staircase method.

Frequency (kHz)	Threshold (dB *re* 20 $\mu Pa^2 \cdot s$) RMS	Standard deviation (dB *re* 20 $\mu Pa^2 \cdot s$) RMS	Threshold (dB SPL *re* 20 μPa)	No. of reversals	Total no. of trials
0.25	44.5	3.3	47.5	23	137
0.50	34.5	2.6	37.5	28	130
1.0	27.8	2.7	30.8	24	132
1.50	35.3	1.0	38.3	32	133
2.0	39.6	4.9	42.6	52	231
3.0	26.1	2.2	29.1	25	133
4.0	26.8	2.9	29.8	24	139
6.0	10.9	2.3	13.9	28	142
8.0	8.1	2.4	11.1	32	162
10.0	12.8	3.0	15.8	27	139
12.0	10.1	1.2	13.1	25	137
14.0	23.1	2.4	26.1	33	157
16.0	24.3	2.4	27.3	30	134
18.0	27.7	3.6	30.6	28	137
20.0	25.0	3.6	28.0	29	141
22.0	25.6	3.7	28.6	28	135
25.0	29.3	2.0	32.3	28	137
30.0	39.9	2.9	42.9	27	132

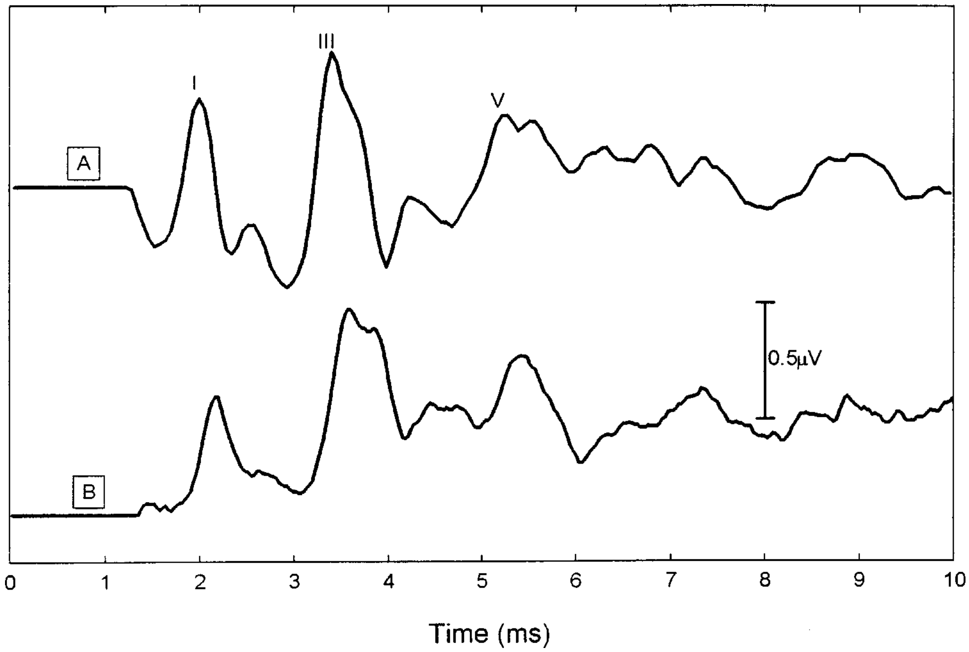

FIG. 2. ABR waveforms generated with (a) clicks and (b) tone-bursts.

testing blocks were discarded due to high false alarm rates as well (only 4 of 100). Standard deviations for the SM method were consistently smaller compared to the MCS method (±2.7 dB *vs* ±5.4 dB).

B. ABR data

ABRs stimulated by both clicks and tone bursts had similar waveforms, with three large peaks (PI, PIII, PV) and two smaller peaks (PII, PIV; Fig. 2). At high intensities, the ABR PI latency was 2 ms and PV latency was 5 ms. The largest waveform peak, PIII, had a maximum amplitude of 0.98 μV, and occurred approximately 4 ms after the tone onset at high intensities. Latencies of the ABR peaks increased with decreasing amplitude, as expected (Hall, 1992).

Thresholds could not be obtained at 8 and 16 kHz due to high ambient noise levels at the time these frequencies were tested (14 dB at 8 kHz, 12 dB at 16 kHz). Thus, points on the ABR audiogram at 8 and 16 kHz are best viewed as the lowest response intensities that could be measured before test stimuli were masked by ambient noise.

C. Comparison of ABR and SM audiograms

The more thoroughly tested behavioral hearing curve (SM) was compared to the ABR curve (Fig. 3). At 2 and 4 kHz, ABR thresholds were 5.3 and 5.1 dB less sensitive than behavioral thresholds, respectively. At 22 kHz, the ABR threshold was less sensitive than the behavioral threshold, although the difference was small, 3.0 dB. ABR thresholds

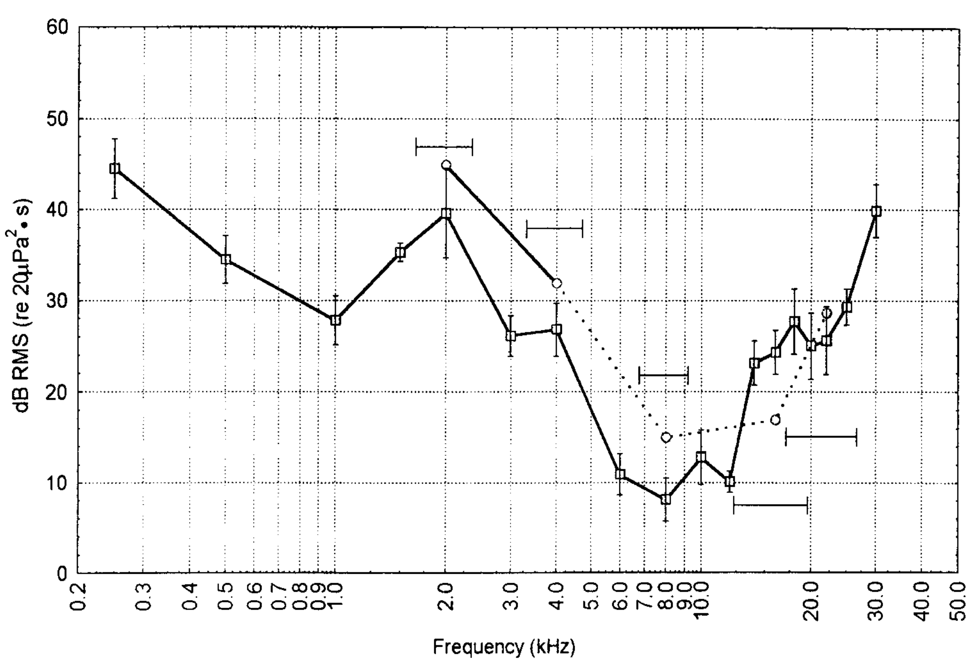

FIG. 3. Comparison of audiograms generated by the DS method (squares) and ABR method (circles). Horizontal range bars indicate the frequency bandwidth of ABR stimuli at threshold. The points on the ABR curve at 8 and 16 kHz are not threshold values; they are the lowest intensities in which a positive ABR was generated before the test stimuli dropped into the noise floor.

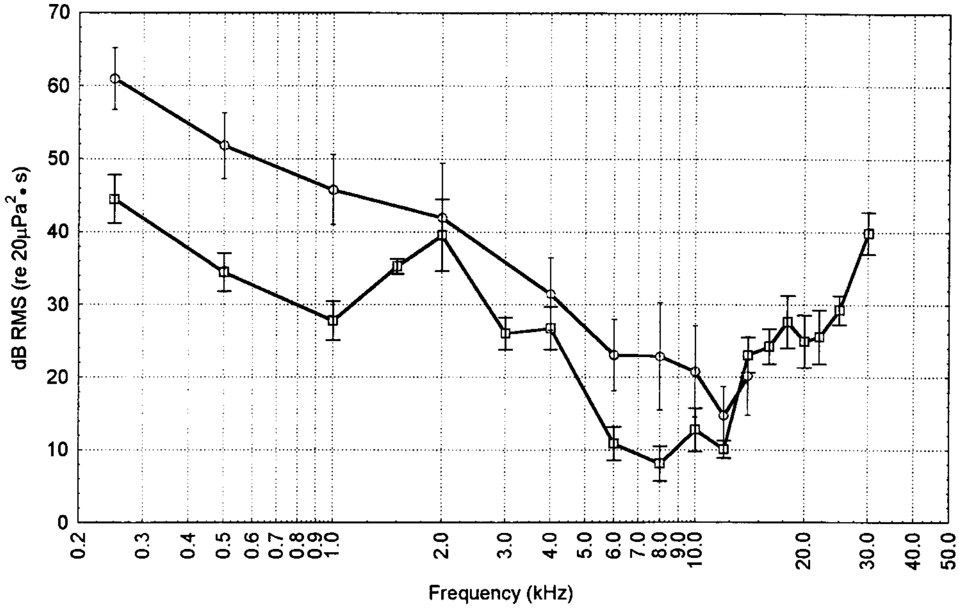

FIG. 4. Comparison of audiograms generated by the DS method (squares) and MCS method (circles).

were not obtained at 8 and 16 kHz. PIII was detectable at the lowest amplitudes tested, 14.9 and 16.9 dB, respectively; at 16 kHz, the lowest amplitude at which PIII was detectable was at least 7.4 dB lower than the behavioral threshold (24.3 dB).

IV. DISCUSSION

A. Comparison of behavioral audiograms

Both behavioral methods employed in this study yielded audiograms of similar form, with best sensitivities ranging between 6 and 12 kHz (Fig. 4). Across the range of frequencies tested, the SM method yielded lower threshold estimates and smaller standard errors. In addition, the SM method yielded a higher measure of sensitivity (d': 2.318 versus 2.201 for the MCS). Minimum differences between the two methods ranged from 1 to 4 dB and the largest differences ranged from 15 to 20 dB.

Several factors may explain the higher sensitivity of the SM method. First, audiometric testing that employs an adaptive up–down procedure yields lower threshold estimates than simple up–down methods or random stimulus presentation (Levitt, 1970). It has been suggested that patterned stimulus presentation increases the attention and concentration of the test subject, resulting in the detection of stimuli at lower amplitudes. The subject in these experiments appeared to be most attentive and motivated when the number of consecutive negative responses (no-go) was minimized, introducing an increase in probability of correct responses relative to the MCS trials. Note that this bias was not a result of an increase in false positive responses, which were kept low in both sets of experiments by terminating trial blocks with too many incorrect catch trial responses.

Second, it may be that subjects can process a predictable stimulus more easily than an unpredictable one, particularly if given feedback during trials (Zwislocki *et al.*, 1958). To reduce false positive responses, we reduced predictability by varying latency to tone presentation, varying initial stimulus levels, and introducing catch trials unpredictably. However, the seal could still be sure that (1) the first tone actually presented in a trial block would be audible, and (2) that a reversal would occur within a few trials. There may have been a greater motivation to perform in SM versus MCS trials if the seal perceived a task with a greater proportion of detectable tones as "easier" or more rewarding.

A third factor could have been the change in reinforcement schedule between paradigms. During the MCS experiment, the seal was reinforced both for responding correctly to the presentation of a tone (moving to the YT) and for remaining on the RT during catch trials. The average false response rate using this reinforcement schedule was 8.7% (97 of 1111 trials). During SM testing, the seal was reinforced only for responding correctly to a tone (moving to the YT). This pay-off schedule change was expected to result in a lower false response rate, as the animal adopted a stricter criterion for making only correct responses. After this change in protocol, the average false response rate dropped (5.6%, 145 in 2588 trials) and there was a decrease in the number of trial blocks with a false response rate above 10% (16% during MCS trials versus 4% during SM trials). During SM trials the seal often remained motionless on the RT for long periods (20–40 s), only moving off the target when a tone was given or when the trainer signaled the end of the trial block, as expected given the change in reinforcement schedule and subsequent change in response bias. In contrast, during MCS trials the seal moved frequently during intertrial intervals and did not remain on the RT for extended periods.

The behavioral audiograms obtained during this study were consistent with those of Mohl (1968), Terhune and Turnbull (1995), and Kastak and Schusterman (1998). The thresholds between 6 and 12 kHz, to our knowledge, are the lowest reported to date for a harbor seal. The audiograms show a high frequency roll-off similar to that reported by Mohl (16–22 kHz), but at higher frequencies (22–30 kHz). Both observations suggest that the subject of these experiments had better hearing than other harbor seals that have been tested.

An alternate explanation for the low threshold measurements between 6 and 12 kHz is that the seal might have taken advantage of standing wave patterns in the test chamber by positioning herself in an area where signal strength was maximized by reflections. This seems doubtful, as the seal's head was positioned in an area that was precisely mapped prior to testing. The seal did not exhibit any behaviors consistent with a search for a ''sweet spot.'' The multiple-speaker setup reduced reflections of test signals, thereby reducing the strength of any standing waves. Test stimuli in the immediate area around the head had a signal strength variance of $<\pm 2$ dB, meaning that any standing waves increased signal amplitude by no more than 2 dB, within the error of the threshold measurements.

B. Stimulus and procedural issues

The behavioral and ABR audiograms had similar shapes. However, the SM method produced lower thresholds than ABRs at mid-range frequencies (3–8 kHz) while the ABR method produced a lower threshold than the SM method at 16 kHz. The SM method was more sensitive at 22 kHz, although by a smaller margin than at lower frequencies. These differences in measured sensitivity between the methods may be the result of stimulus characteristics, as well as the testing environment and activity of the subject during testing.

1. Stimulus characteristics

Stimulus characteristics affect the results of physiological and behavioral testing. In mammals, behavioral thresholds are often higher for tones with durations shorter than the integration time of the ear (Watson and Gengel, 1969), consistent with the extensive knowledge of how integration time affects sound perception (Yost, 1994). This is why behavioral studies are typically performed with stimuli lasting between 250 and 500 ms. These longer stimuli also have relatively narrow bandwidths, less than 50 Hz for 500-ms tones.

In contrast, ABR stimuli must be brief to elicit good response waveforms, usually less than 5 ms. Hall (1992) recommends using five-cycle stimuli in a 2–1–2 pattern (rise–plateau–fall) for ABR testing. The resulting time waveforms have wider bandwidth than tone pips used in behavioral studies. A five-cycle ABR waveform centered at 8 kHz is 0.6 ms in duration and has a bandwidth of 2 kHz at or near threshold level. Thus, in the current study, behavioral responses were stimulated using narrow-band stimuli and ABR responses were stimulated using wideband stimuli simply as a result of differences between the two techniques. The frequency bandwidths of ABR stimuli near observed thresholds are shown in Table III. This difference may account for some of the observed differences in threshold estimates between the two methods. Generally, broader-band ABR stimuli correlate with more robust responses because larger portions of the cochlea are excited by wideband stimuli (Hall, 1992).

As an example, in our study the ABR threshold at 16 kHz was estimated to be at least 16.9 dB and the SM behavioral threshold was 24.3 dB. The bandwidth of the ABR stimulus at threshold was approximately 7.4 kHz centered at 16 kHz, while the bandwidth of the behavioral stimulus at 16 kHz was less than 25 Hz (Fig. 5). Thus, the 16 kHz ABR test signal would have stimulated the cochlea with frequencies as low as 13 kHz. The contribution of neurons tuned to this frequency could easily explain the greater sensitivity of the ABR response observed at 16 kHz.

TABLE III. Frequency bandwidth of ABR stimuli at threshold.

Center frequency (kHz)	ABR threshold (dB *re* 20 μPa$^2\cdot$s) RMS	Frequency bandwidth at threshold (kHz)
2	44.9	0.7
4	31.9	1.4
8	~15.0	2.4
16	~16.9	7.4
22	28.6	9.4

2. Testing environment and subject activity

Myogenic noise resulting from muscle activity interferes with ABR test sensitivity, which is recognized as a major contributor to the lower sensitivity of ABR measurements (Hall, 1992). Human infants commonly fall asleep during ABR testing, resulting in better signal-to-noise ratio and thus clearer and more sensitive ABR measurements (Sininger, 1993). During this study, ABR stimuli were presented in order of ascending frequency. The seal was alert during the first half of the ABR testing period, but approximately halfway through testing (near the completion of data collection at 8 kHz) she became deeply relaxed and remained relatively motionless. The relaxed state was evidenced by lower heart rate and muscle activity observable from EEG and EMG traces, and a reduction in stimulus artifacts reported by the Bio-Logics system. The decrease in myogenic noise while testing at 16 and 22 kHz appears correlated with estimated thresholds lower than those yielded by SM measurements at 16 and 22 kHz. Thus, the seal's reduced activity during high-frequency testing may have contributed to lower threshold estimates at these frequencies. This relationship has been observed during audiometric testing of elephant seals and sea lions (Bowles, unpublished data); ABRs had better morphology and amplitude when test animals were quiescent, making thresholds easier to measure.

C. Summary

In summary, the two audiograms resulting from behavioral and ABR testing of an individual harbor seal agreed well, indicating that ABR measurements, which can be collected in a single day from an untrained animal, can produce results comparable to six months of behavioral testing. The results must be interpreted with caution because the frequency bandwidths of ABR stimuli can affect sensitivity and threshold estimates. In future studies of this kind, ABR stimulus characteristics should be varied systematically to obtain a more refined test signal, one that yields a clear ABR but has the minimum possible bandwidth.

For absolute threshold determination at discrete frequencies, behavioral methods remain the most accurate method. However, with careful selection of ABR stimuli and control of the testing environment, hearing sensitivities can be measured for a much larger number of individuals than is usually

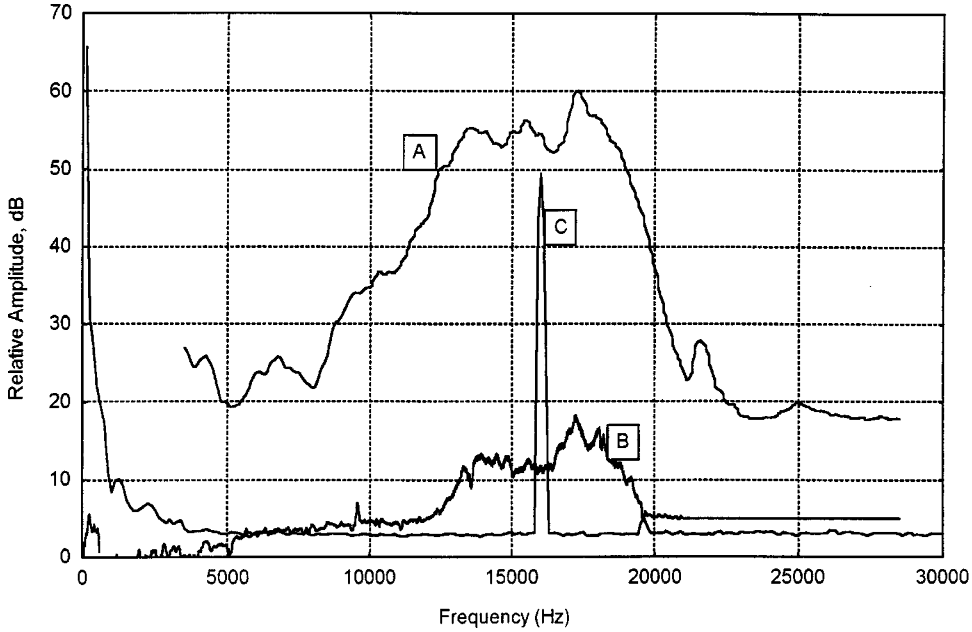

FIG. 5. Comparison of ABR and behavioral stimuli at 16 kHz. (a) 16-kHz ABR stimuli at high amplitudes. (b) ABR stimuli near recorded threshold; note the bandwidth at this point is approximately 6 kHz, ranging from 13 to 16 kHz. (c) 16-kHz behavioral stimuli at high amplitude. Bandwidth did not change appreciably as amplitude was lowered. Horizontal range bars indicate the frequency bandwidth of ABR stimuli at threshold.

possible using behavioral testing methods. Given the difference in sensitivity between our test subject and other subjects in previous experiments, it is likely that significant variation in hearing ability will be found across individuals in pinniped populations, just as in humans and laboratory animals. Once perfected, ABR measurements could be used to obtain much needed data on population-level characteristics of pinniped hearing.

ACKNOWLEDGMENTS

Thanks to the Wild Arctic Animal Care Team of SeaWorld San Diego. Erik Berg made important contributions such as designing the sound isolation box and performing acoustic calibrations. Carolyn Schlundt at SPAWAR in San Diego and Yvonne Sininger at the House Ear Institute provided technical insight and helpful discussions. Two anonymous reviewers offered valuable comments on drafts of the manuscript. Special thanks to Kevin Shepard at NASA/Langley for his staunch support and patience throughout this study. Funding was provided by a NASA Research Grant No. NAS1-20101 and a generous grant from the Joan Irvine Smith and Athalie R. Clarke Foundation. The work was permitted by the National Marine Fisheries Service (No. 872-1397).

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