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Auditory Evoked Potentials in Northern Elephant Seals (*Mirounga angustirostris*)

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Abstract

Auditory evoked potentials (AEPs) were investigated in northern elephant seals (*Mirounga angustirostris*) to characterize the responses elicited by different acoustic stimulus types, examine temporal resolving capabilities, and evaluate the potential for using evoked responses to estimate hearing sensitivity. Clicks and tone pips were presented to individual seals to characterize evoked responses to broad- and narrowband stimuli. Tone pip trains and sinusoidally amplitude-modulated (SAM) tones were used to determine modulation rate transfer functions (MRTF) of the auditory system and to determine if the magnitude of the envelope-following response (EFR) relative to the stimulus level can be used to estimate hearing thresholds. Click evoked responses were characterized by three early positive peaks (~2.6, 4.4, and 6.1 ms) and a dominant negative peak at 7.2 ms and had average amplitudes of 264 nV (peak-to-peak [pk-pk]) for a corresponding stimulus level of 126 dB re 20 µPa (pk-pk). The use of dissociative drugs for the immobilization of the seals showed no demonstrable effect on the latencies or amplitudes of the click evoked response. Both the rate following response (RFR) and EFR amplitudes were maximal when the stimulus repetition rate or the amplitude modulation rate, respectively, were < 100 Hz. EFR amplitudes at the rate of amplitude modulation tracked near linearly with stimulus level. Thresholds for a 4-kHz SAM tone were estimated to be 45 dB re 20 µPa. Thus, the recording of AEPs is a viable means of studying auditory processes in the northern elephant seal.

Key Words: elephant seal, audiometry, evoked potential, hearing

Introduction

Evoked potential audiometry has enjoyed great success in its application to the study of odontocete hearing (see Supin et al., 2001, for an overview). Much of this success is because of the robust neurological response of the odontocete auditory system to acoustic stimuli, a response that exists because of a large auditory nerve, thin skull (relative to many other mammals of comparable size), and smooth skin that permits the relatively easy attachment of surface electrodes for measurement of evoked potentials (Ketten, 1994, 1997; Supin et al., 2001). Other marine mammals, primarily the pinnipeds and mysticete cetaceans, have auditory nerves that are proportionally smaller relative to odontocetes, have thicker skulls, and can exceed the mass of odontocetes by orders of magnitude (Leatherwood & Reeves, 1983; Ketten, 1994, 1997). One or more of these factors contribute to an increased difficulty in obtaining evoked potentials in non-odontocete marine mammals (Ridgway & Carder, 2001).

The development of AEP techniques for large mammals will benefit from the progressive adaptation of techniques to animals of increasing mass. The northern elephant seal (*Mirounga angustirostris*) is the largest of the phocid seals in the northern hemisphere, covering a range of masses from birth to adulthood of ~35 kg to in excess of 2,000 kg (Kretzmann et al., 1993; Deutsch et al., 1994), respectively. The northern elephant seal develops a thick dermis and blubber layer as well as a thick, ossified skull, which in concert with the seal's large mass contribute to the attenuation of evoked potentials at the surface of the skin. These issues are present to some extent in all pinniped species, and prior electrophysiological studies on pinnipeds used invasive techniques (e.g., skull implants) to

increase the signal-to-noise ratio of neurological signals (Alderson et al., 1960; Bullock et al., 1971; Ridgway & Joyce, 1975). These studies were the first to characterize the auditory centers in the harbor seal (*Phoca vitulina*) (Alderson et al., 1960) and sea lion (*Zalophus californianus*) (Bullock et al., 1971) and the auditory evoked responses to click and tone pip stimuli in harbor seals, grey seals (*Halichoerus grypus*), and sea lions (Alderson et al., 1960; Bullock et al., 1971; Ridgway & Joyce, 1975). Preliminary attempts to estimate auditory sensitivity yielded promising results. Although absolute sensitivities were not the same, the shape of the auditory threshold functions appeared similar to those obtained behaviorally in the same and confamilial species (Møhl, 1968; Bullock et al., 1971; Schusterman, 1974; Ridgway & Joyce, 1975). Recent work with subcutaneous electrodes in harbor seals demonstrated the feasibility of obtaining click and tone pip-evoked responses via subcutaneous electrodes and the potential for estimating auditory sensitivity with AEPs (Wolski et al., 2003). Further application and refinement of evoked potential methods in pinnipeds, with the goal of determining the potential for utilizing frequency specific AEP techniques, is therefore warranted.

The northern elephant seal is easily accessible along the western coast of the United States and provides a model system within which to adapt AEP techniques to animals of progressively larger mass. The goal of the present study was to determine whether AEP methods could be applied to the northern elephant seal. To this end, investigations were undertaken under both laboratory and field conditions and with different age classes of seal. The study focused on the smaller seals (weanlings and 1.8-y-old animals) in order to first determine the feasibility of obtaining AEPs. Evoked responses were recorded from click stimuli, tone pips and tone pip trains, and sinusoidally amplitude-modulated (SAM) tones. The latter response, known as the envelope-following response (EFR), is an evoked response that is locked to the envelope of the SAM tone. The relationship between EFR amplitude and SAM tone modulation frequency (the modulation rate transfer function [MRTF]) was also assessed. The feasibility of obtaining AEPs in elephant seals is discussed with emphasis on characteristics of the evoked response and its relation to methodological considerations.

Materials and Methods

Several tests were performed to characterize the electrophysiological response to different types of acoustic stimuli and the impact of chemical immobilization on evoked responses. Stimuli consisted of clicks, tone pips, and SAM tones. Clicks are transient signals with broad spectral content often used to test hearing sensitivity across a range of frequencies simultaneously. Tone pips are short bursts of a tone, typically containing only a few cycles and with durations less than a few milliseconds, which have a narrower spectral content than clicks and presumably excite a smaller region of the basilar membrane. SAM tones are tones which are amplitude modulated, to some degree, according to a sine function. SAM tones have a narrow stimulus spectrum and have been applied to odontocete cetaceans to investigate temporal resolving capability and auditory sensitivity to narrowband stimuli (Dolphin et al., 1995; Supin & Popov, 1995; Dolphin, 2000; Klishin & Popov, 2000; Supin & Popov, 2000; Yuen et al., 2005; Finneran & Houser, 2006; Houser & Finneran, 2006; Mooney & Nachtigall, 2006). The sinusoidal envelope elicits a rhythmic evoked response that can be analyzed in the frequency domain and which provides greater frequency specificity than tests measuring evoked responses to either clicks or tone pips.

Subjects

Audiometric evaluations were conducted on elephant seals either at Long Marine Laboratory (LML) at the University of California–Santa Cruz or at the Año Nuevo State Reserve in California. All elephant seals selected for the audiometric testing were between 1.3 and 1.8 y of age (age estimates were based upon the time of year the seals are on land, size, and lack of secondary sexual characteristics). Tests were conducted between the spring of 2005 and the summer of 2006 and were performed on ten subjects. Not all of the results are presented here.

One seal was manually captured at Año Nuevo, placed in an aluminum transport cage, and transported by truck to LML. This seal was used to determine the impact of chemical immobilization on AEPs and was therefore manually restrained on a plastic restraint board for initial testing (see below). Tests were conducted in a hemianechoic chamber with background noise levels below 10 dB re 20 μPa for frequencies ≥ 500 Hz (Holt et al., 2004). Procedures on this seal lasted approximately 5 h, but included studies that are not reported here. The seal was released at Año Nuevo within 24 h of capture.

All other seals were studied at Año Nuevo. These seals were immobilized with 1.0 mg/kg of tiletamine HCl/zolazepam HCl and were not submitted to any additional restraint (i.e., no restraint board was used). Immobilization was maintained with 0.5-cc intravenous injections of ketamine as needed (Briggs et al., 1975). Injections of

diazepam (0.5 cc) were administered intramuscularly as needed to control for tremors resulting from ketamine immobilization. Procedures conducted on seals at Año Nuevo ranged in time from 3 to 5 h, but entailed more tests than are presented in this manuscript. Ambient noise at Año Nuevo was measured during each of the test periods using a portable sound-level meter (B&K 2239). Noise levels at Año Nuevo were dominated by wind and the sounds of nearby animals; levels were typically from 50 to 60 dBA.

AEP Recordings

For the experiments at LML, a personal computer (PC) with a multifunction data acquisition card (National Instruments PCI-MIO-16E-1) was used to generate sound stimuli and digitize and record the evoked responses. Stimuli were created at 12-bit resolution and a 500-kHz update rate, attenuated (Tucker-Davis Technologies PA-5), bandpass filtered (Krohn-Hite 3C Module, 1 to 150 kHz), and amplified (Hafler P1000) before being delivered to headphones. At Año Nuevo, a rugged notebook computer with a multifunction data acquisition card (National Instruments PCI-6251) was used to generate stimuli at a 2-MHz update rate and with 16-bit resolution. Sounds were low-pass filtered (20 kHz, Krohn-Hite 3C Module) and passed through a custom attenuator before delivery to the headphones.

Sounds were presented to the subjects using either TDH-39 (Telephonics Corp.) or Bose 2 Acoustic Noise Cancelling (Bose Corp.) headphones. Headphones were placed over the external meatus of the subjects. At the start of each session, the stimuli (clicks, tone pips, and SAM tones) were calibrated with an Etymotic probe microphone (sensitivity of 50 mV/Pa). Peak-to-peak (pk-pk) sound pressure was measured for clicks while the root mean squared (rms) sound pressure was measured for tone pips and SAM tones. Clicks consisted of either 100 or 200 μs rectangular waveforms with no rise or fall time. Tone pips consisted of five cycles: a two-cycle linear rise time, 1 cycle at full amplitude, and a two-cycle linear fall time (2-1-2 pip). The duration of the tone pips depended on the frequency of the tone. SAM tones were generated with 1-ms cosine rise and fall times and were amplitude modulated at variable frequencies. Unless noted otherwise, the polarity of the stimulus was sequentially alternated in order to cancel out any artifact introduced into the AEP recordings.

Subcutaneous stainless steel needle electrodes (Neuroline, 1.7-cm needle, 50- or 100-cm lead wires) were used for the detection of evoked potentials (Figure 1). For all seals, the non-inverting (+) electrode was inserted on the dorsal midline of the head, equidistant from the left and right external ears, or 2 cm in front of this position on the midline. The maximum amplitude of the evoked response varied from seal to seal but was maximal within these limits as was determined from prior exploratory tests. The inverting electrode (-) was placed 5 cm below and 7 cm behind either the right or the left external meatus. The ground electrode was placed on the back of the seal, approximately at the longitudinal insertion of the pectoral flippers. Once inserted, an impedance check was made to ensure that the impedance difference across all of the electrodes was less than 5 kΩ. Electrode signals were differentially amplified and filtered using a biopotential amplifier (Grass ICP-511). The biopotential amplifier gain was fixed at 100,000. Unless otherwise noted, high- and low-pass filters varied from 100 to 300 Hz and 100 Hz to 1 kHz, depending on the stimulus modulation frequency and measurement sampling rate. The resulting signal was digitized using the PCI-MIO-16E-1 or PCI-6251. Recording sampling rates, recording durations, and stimulus durations varied as a function of the test being conducted (see below).

Figure 1. Schematic of subcutaneous electrode placement on the head of the northern elephant seal; the positive symbol corresponds to the non-inverting electrode, the negative symbol corresponds to the inverting electrode, and GND corresponds to the ground electrode.

Impact of Immobilization

A 1.3-y-old male seal was captured and transported to LML on 3 March 2005. The seal (03MAR05) was manually restrained with seatbelts on a plastic board for the placement of subcutaneous electrodes. The seal was then placed within the hemi-anechoic

chamber. The seal was manually restrained for initial click evoked potential measurements and was then immobilized with an intramuscular injection of tiletamine/zolazepam (1.0 mg/kg). Complete immobilization takes ~10 min following tiletamine/zolazepam injection. During this period, several measurements of the click evoked response were made. Following the initial immobilization, immobilization was maintained with intravascular administration of ketamine, as needed, via the extradural vein (Briggs et al., 1975).

Click evoked potentials were collected across an approximately 1.5-h period and were recorded every 2 min following the complete immobilization from tiletamine. Clicks consisted of 100 µs rectangular waveforms with no rise or fall time and were presented at a rate of 20 Hz. The pk-pk sound pressure level (SPL) was held constant at 148 dB re 20 µPa. A total of 1,000 averages were collected for each stimulus presentation with an evoked potential recording window of 50 ms. The artifact reject level was set at 10 µV for all collections, and the biopotential amplifier high- and low-pass filters were set to 100 Hz and 3 kHz, respectively. Since this procedure occurred early in the development of the stimulus control software, the polarity of the clicks was not alternated on successive click presentations.

Click Evoked Response

A 1.8-y-old female elephant seal (10MAR06A) was immobilized at Año Nuevo on 10 March 2006 to determine the waveform characteristics of click evoked responses. Clicks were presented at a rate of ~39 Hz, the evoked response recording window was 26 ms, responses were digitized at 10 kHz, and 4,000 averages were collected for each stimulus presentation. Two stimulus presentations were performed using a click pk-pk level of 126 dB re 20 µPa. The artifact rejection level was set at 8 µV, and the high- and low-pass filters were set at 100 Hz and 1 kHz, respectively.

Tone Pips and Tone Pip Trains

A 1.8-y-old male seal (15AUG06A) was immobilized on 15 August 2006 for the study of tone pip-evoked responses. Multiple variations on the stimulus presentation were performed to determine the characteristics of the tone pip-evoked response and the feasibility of using tone pip trains to estimate auditory sensitivity. Testing parameters consisted of the following:

- Single 2-1-2 pips were generated using a 2-kHz center frequency (f_c) (output duration of 2.5 ms). Tone pips were presented at a rate of 33 Hz; the evoked response recording window was set to 30 ms, responses were digitized at 10 kHz, and 2,000 averages were collected for the stimulus presentation. The stimulus level was set at 100 dB re 20 µPa, and the artifact rejection level was set at 8 µV.
- A series of 10 tone pips were used to create a tone pip train. Tone pips were 2-1-2 pips with f_c of either 2 or 4 kHz (output durations of 2.5 and 1.2 ms, respectively). Trains were presented such that there was a 10-ms delay between the onsets of successive tone pips. Evoked response recordings were 100 ms in duration, and 4,000 averages were acquired for each stimulus presentation. Bandpass filters were set at 0.1 to 1 kHz, and evoked responses were digitized at either 2 or 6 kHz. The artifact rejection level was set at 8 µV for all stimulus presentations. For the 2 kHz tone pips, the stimulus level was set at 100 dB re 20 µPa for the initial stimulus presentation and decreased by 10 dB for the second presentation. Stimulus level was then sequentially reduced by 5 dB on consecutive presentations. For the 4 kHz tone pips, the stimulus level was set at 95 dB re 20 µPa for the initial stimulus presentation and decreased by 10 dB for the second presentation. Stimulus level was then sequentially reduced by 5 dB on consecutive presentations. A total of eight stimulus presentations were conducted with the 2-kHz tone pips, and a total of ten were conducted with the 4-kHz tone pips.
- Three series of pip trains with different stimulus presentation rates were used to investigate the rate following response (RFR), the relationship between the evoked response amplitude and the rate at which stimuli are presented. Each tone pip train consisted of a series of 2-1-2 pips with a 2-kHz center frequency. The repetition rate of the tone pips was varied at 200, 300, and 400 Hz, and recording windows were set at 55, 38, and 30 ms. All tone pips were presented at 100 dB re 20 µPa, and 2,000 averages were acquired for each stimulus presentation.

MRTF and the EFR

A 1.8-y-old male seal (16AUG06B) was immobilized on 16 August 2006 to investigate the MRTF of the northern elephant seal. A 100-ms duration SAM tone with a 4-kHz carrier was used to test the evoked response amplitude as a result of SAM tone modulation rate. Modulation rates of 80 to 1,000 Hz were used. SAM tones were presented at stimulus levels of 113 dB re 20 µPa. The bioamplifier high- and low-pass filters were set at 0.03 to 1 kHz (80-Hz modulation rate), 0.1 to 1 kHz (100- to 900-Hz modulation rate), or 0.1 to 3 kHz (1,000-Hz modulation rate). The data acquisition scan rate was either 2 kHz (80- to 900-Hz modulation rate) or 6 kHz (1,000

Hz). The measured AEP amplitudes and phase angles were corrected for the frequency response of the bioamplifier filters and the 6-ms latency between the stimulus onset and the analysis window start.

A 4-kHz carrier SAM tone with a 200-Hz modulation rate was used as a stimulus to determine the feasibility of tracking the amplitude of the EFR as a possible means of using the method to estimate auditory thresholds. Stimuli were 100 ms in duration, and the response recording window for each epoch was set to ~111 ms. A total of 4,000 averages were collected for each presentation of a particular stimulus level. Stimuli were presented at 113 dB re 20 μPa and reduced on successive stimulus presentations by 10 dB; a total of 7 stimulus presentations were made covering a stimulus-level range of 53 to 113 dB. Bioamplifier high- and low-pass filters were set at 100 Hz and 1 kHz, respectively. Evoked responses were recorded with a scan rate of 2 kHz.

Magnitude-squared coherence (MSC) was calculated during each measurement and used to objectively determine if the measured AEP component at the modulation frequency was statistically different from noise (Dobie & Wilson, 1989, 1996; Dobie, 1993). MSC is a ratio of the power (at a single frequency) contained in the "grand" coherent average to the average of the powers within individual "segments" or "subaverages" of the total data stream. The MSC provides a ratio of the signal power to signal-plus-noise power and varies from zero (all noise) to one (all signal). The MSC calculation used 20 subaverages. Critical values for MSC, using α = 0.01, were obtained from Amos & Koopmans (1963) and Brillinger (1978). If the calculated MSC was greater than the critical value, the AEP at the modulation frequency was considered to be detected (see Finneran et al., this issue, for discussion of the MSC relative to other objective response techniques). This process of objective response detection provided a "yes/no" result for each AEP measurement and permitted adaptive procedures for adjusting stimulus levels (e.g., modified staircase technique).

Following data collection, a linear regression was applied to all of the detected responses. The SPL value corresponding to the 0 V crossing of the regression line was then used as an extrapolated threshold value for the frequency tested. Similar processes have been used in the estimation of hearing sensitivity in humans (Campbell et al., 1977) and odontocete cetaceans (Supin et al., 2001; Popov et al., 2005; Yuen et al., 2005; Finneran & Houser, 2006; Houser & Finneran, 2006).

Results

Impact of Immobilization

The click evoked response waveform for a single seal with progression in immobilization from mechanical restraint, to initial immobilization with an intramuscular injection of tiletamine/zolazepam, and maintained immobilization with bolus intravenous injections of ketamine and/or diazepam is shown in Figure 2. The drugging schedule is time-lined according to the waveforms on the right vertical axis. The drugging schedule is the same as that typically used for the immobilization of elephant seals in the wild. (Note that data plotting begins at 2.5 ms to avoid stimulus artifact that was observed between 0 to 2 ms; artifact was removed by sequentially reversing the polarity of the stimulus in all subsequent tests.) Amplitudes and latencies of click evoked responses showed no variation as a function of the immobilization technique. Both were similar regardless of the mode, onset, or duration of the immobilization method.

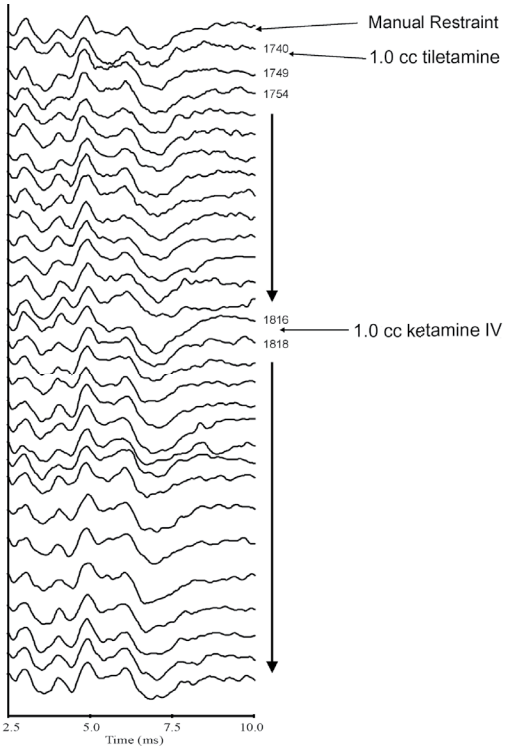

Figure 2. The temporal sequence of click evoked waveforms prior to and following chemical immobilization are plotted in a descending sequence. Numbers to the right of the waveforms correspond to time. Unless otherwise noted, the separation between click waveforms following immobilization is 2 min. Times during which the seal was manually restrained or when tiletamine or ketamine were administered are indicated with arrows.

Click Evoked Response

The click evoked waveform of the northern elephant seal is characterized by three early, positive peaks (~2.6, 4.4, and 6.1 ms) following stimulus onset (Figure 3). A minor positive peak, or ripple, was also observed at ~5.4 ms following stimulus onset. A pronounced negative peak, the most notable characteristic of the click evoked waveform, occurred at 7.2 ms following stimulus onset. The numbering of the click evoked waveforms used for humans is not instituted here since there is no indication that a direct correspondence in waveforms should exist. Therefore, the dominant peaks are identified in order as P1, P2, P3, and N4. Pk-pk amplitude of the waveform, corresponding to the difference between the P3 and N4 amplitudes, averaged 264 nV.

Tone Pips and Tone Pip Trains

The response evoked from the 2-1-2, 2-kHz tone pip is depicted in Figure 4. The evoked waveform is grossly similar to that evoked by the 200-µs click with the P2, P3, and N4 peaks being observable

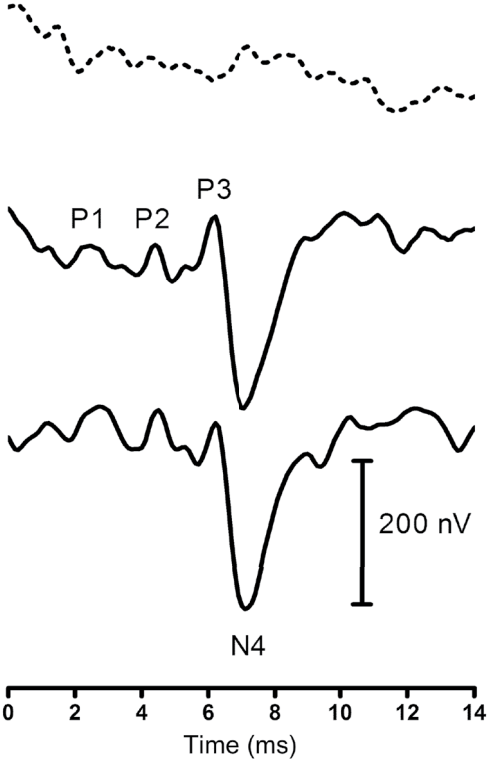

Figure 3. Two collections of the click evoked waveform are presented in relation to the electrophysiological response when no stimulus is present (dashed line). P1, P2, and P3 correspond to positive peaks of the waveform, whereas N4 corresponds to the largest negative peak.

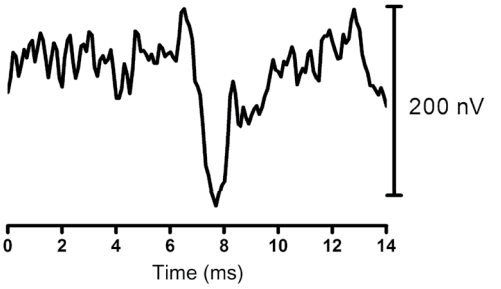

Figure 4. Waveform of the evoked response produced from a 2-kHz tone pip

at comparable latencies. Pk-pk amplitude of the waveform generated by individual tone pips was 211 nV. Figures 5 and 6 show the evoked response waveforms and corresponding spectra produced by trains of 2- and 4-kHz tone pips, respectively. In both instances, periodicity is noted not only at the fundamental repetition rate of the stimulus (100 Hz) but also at harmonics of the repetition rate. Whereas the 200-Hz harmonic is obvious in the 2-kHz tone pip trains, both the 200- and 300-Hz harmonics are noted in the 4-kHz tone pip train, even though the fundamental repetition rate was the same for both series. The relationship between the amplitude of the evoked response at the fundamental (100 Hz) and at the 200-Hz harmonic are demonstrated in Figure 7(A) for the 2-kHz tone pip train and Figure 7(B) for the 4-kHz tone pip train. As the stimulus level decreased, the amplitude of the evoked response also generally decreased at both 100 and 200 Hz. The response curves at 100 and 200 Hz, for both 2- and 4-kHz tone pips, showed marked nonlinearities; however, at the lower stimulus levels of the 4-kHz tone pip train, the response curves approached a linear decline in amplitude with decreasing stimulus level.

The RFR waveform was well defined for both the 200- and 300-Hz presentation rates, but diminished in quality at 400 Hz (Figure 8[A]). RFR amplitudes were 21.2, 17.8, and 4.0 nV for stimulus presentation rates of 200, 300, and 400 Hz, respectively (Figure 8[B]). The 100-Hz rate utilized previously produced an RFR amplitude of 36.0 nV, thus eliciting the maximal response of the presentation rates tested.

MRTF and the EFR

Figure 9 shows the amplitude and phase angles of the EFR for SAM modulation frequencies ranging from 80 Hz to 1 kHz. The pattern of diminishing EFR amplitudes with increases in the modulation rate was similar to that observed for the limited number of RFRs tested. Responses were detected across the range of modulation frequencies tested, and the maximum amplitude corresponded to a modulation frequency of 80 Hz. The range

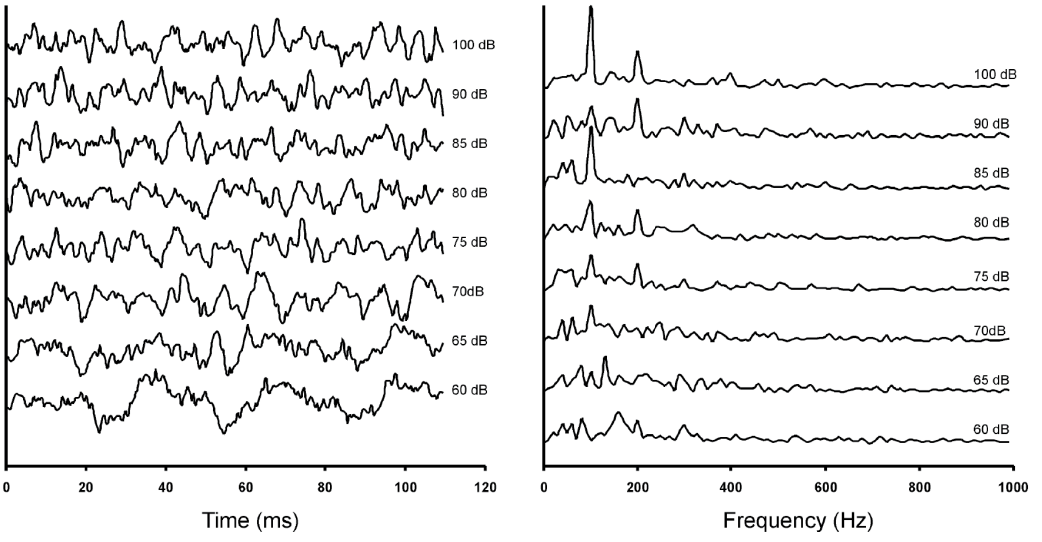

Figure 5. Waveform (left panel) and spectra (right panel) of the evoked response produced from a 2-kHz tone pip presented at a rate of 100 Hz

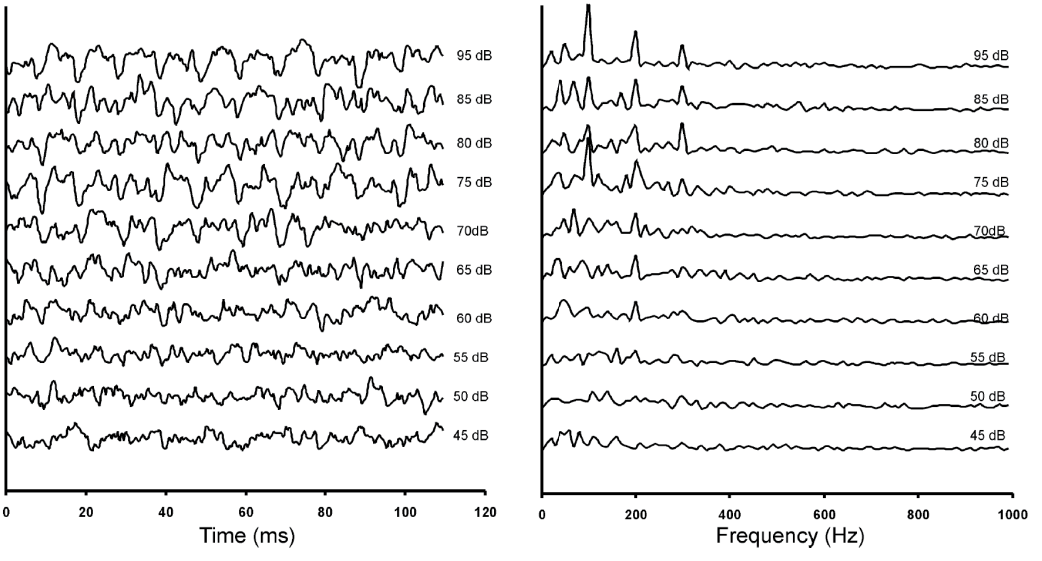

Figure 6. Waveform (left panel) and spectra (right panel) of the evoked response produced from a 4-kHz tone pip presented at a rate of 100 Hz

of EFR amplitudes was relatively narrow (3 to 38.6 nV) across the range of amplitude modulation frequencies tested. For the phase data, a linear regression was performed over the modulation frequency range of 80 to 800 Hz where the data points exhibited good linearity (r^2 = 0.99). The group delay T_d,

$$T_d = \frac{\Delta\theta / \Delta f_m}{2\pi}, \qquad (1)$$

was calculated from slope of the regression line, $\Delta\theta/\Delta f_m$, where the slope is in units of rad/Hz and T_d is expressed in seconds. The group delay calculated from the regression line slope was 2.9 ms.

Figure 10 shows the evoked response waveform and spectra corresponding to each of the different stimulus levels tested with the 4-kHz SAM tone. The amplitude of the evoked response at the modulation frequency declined with stimulus level. As in the RFR tests, spectral peaks were notable not only at the modulation frequency (200

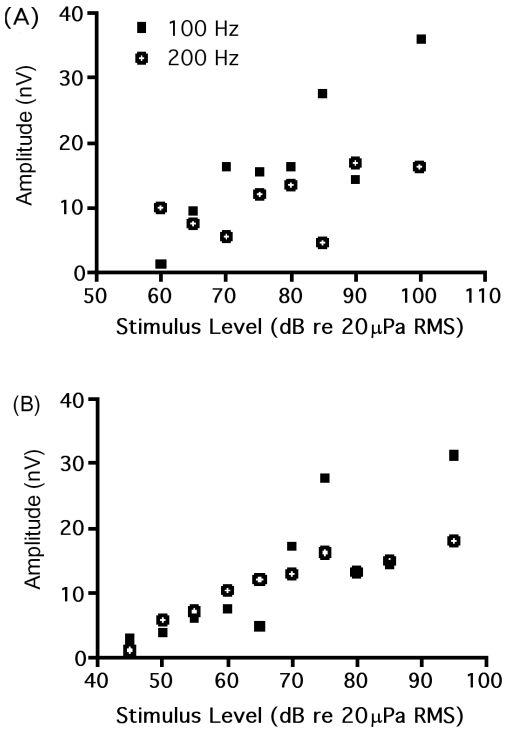

Figure 7. Amplitude of the evoked response resulting from a 2-kHz (A) and 4-kHz (B) tone pip presented at a rate of 100 Hz; filled squares correspond to the amplitude of the spectrum at the fundamental presentation rate, and open circles correspond to the amplitude of the spectrum at the 2nd harmonic (200 Hz).

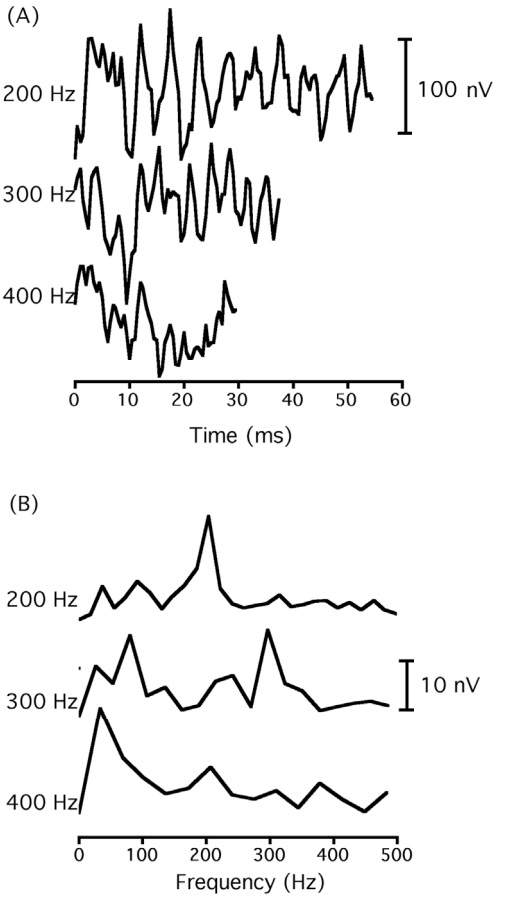

Figure 8. (A) Waveforms of the evoked response corresponding to a 2-kHz tone pip presented at rates of 200, 300, and 400 Hz; (B) spectra of the waveforms presented in (A).

Hz) but also at harmonics of 400 and 600 Hz. Figure 11 demonstrates the change in EFR magnitude with stimulus level. Filled squares denote those evoked responses that were detected using the statistical application of the MSC, whereas open symbols represent those values which were not significantly different from noise. The $r^2 = 0.96$ for the regression line and extrapolation to the 0 V crossing, demonstrated by the dashed portion of the regression line, yielded a threshold estimate for the 4-kHz SAM tone of 45 dB re 20 μPa.

Discussion

The overall amplitudes of click and tone pip-evoked responses and the EFR in the elephant seal are an order of magnitude smaller than those observed in odontocete studies at the maximal stimulus levels tested (Popov & Supin, 1985; Dolphin et al., 1995; Supin & Popov, 1995; Popov & Klishin, 1996; Szymanski et al., 1999). Despite the use of subcutaneous needle electrodes to overcome the attenuation of the evoked response through the dermis, and the high stimulus amplitudes, the resulting evoked response amplitudes were never more than several hundred nV at high stimulus levels. Paramount to measuring these signals was the minimization of physiological and other interfering electrical noise. Under field conditions, the AEP system was run off batteries, and system noise was generally less than 5 nV. It was under these conditions that the highest quality and lowest amplitude AEPs were obtained. By comparison, testing at LML resulted in a substantially elevated noise floor that would likely have precluded the detection of signals that were acquired under field testing conditions. The higher noise levels were likely due to the presence of many electrical sources in the vicinity of the tests as well as the use of AC power to run the AEP system.

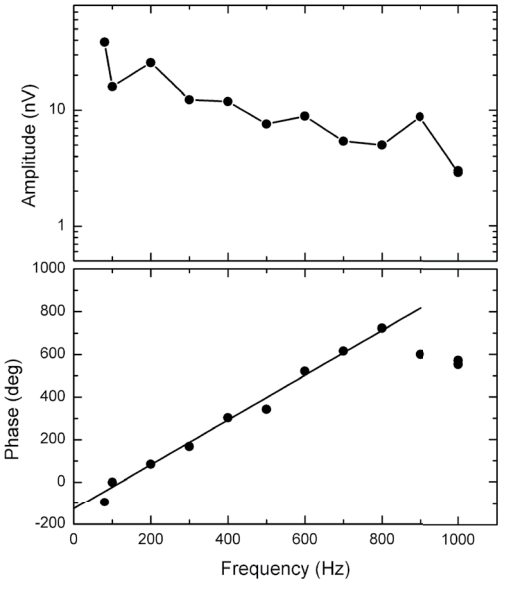

Figure 9. The MRTF (top panel) and phase (bottom panel) of the evoked response corresponding to modulation frequencies from 80 to 1,000 Hz; the solid straight line in the phase plot is produced from a linear regression of the phase data across the modulation frequencies from 80 to 800 Hz. The slope of the line is used in the calculation of group delays.

The nomenclature given the click evoked waveform peaks is different than that used by Wolski et al. (2003), due in part to the fact that the peaks of note in the harbor seal study terminated earlier than those recorded in the elephant seal. Note, however, that there is some ambiguity in the interpretation of the peaks provided by Wolski et al. as the polarity of the peaks is not explicitly given; it is assumed here that the peaks identified are positive. Portions of the click evoked waveform structure obtained in the elephant seal are nonetheless similar to those found in the harbor seal. Specifically, the P1 and P3 waves of Wolski et al. correspond closely to the P2 and P3 waves noted in this study. These peaks are separated by ~2 ms in both species, although the P2 wave of the elephant seal has a latency ~2 ms later than the P1 of the harbor seal, and both have an intervening ripple in the waveform. This ripple was designated as the P2 of the click evoked waveform in the harbor seal. The dominant negative peak present at ~7 ms in the elephant seal does not occur to the same extent in the harbor seal; rather, a positive peak (P3) is dominant in the harbor seal click evoked response.

The increased variability in the tone pip-evoked waveform diminished the presence of the P1 and P2 waves, but the latencies were similar to those elicited by clicks. A difference of ~0.5 ms in the latencies between clicks and tone pips is possibly due to the use of different subjects for the two tests. Lower amplitudes and the narrower spectrum of the tone pips are also likely contributors, however, as each of these has been shown to increase evoked response latencies (Goldstein & Aldrich, 1999).

Tone pip pk-pk amplitudes linearly change with stimulus level in the sea lion (Bullock et al., 1971).

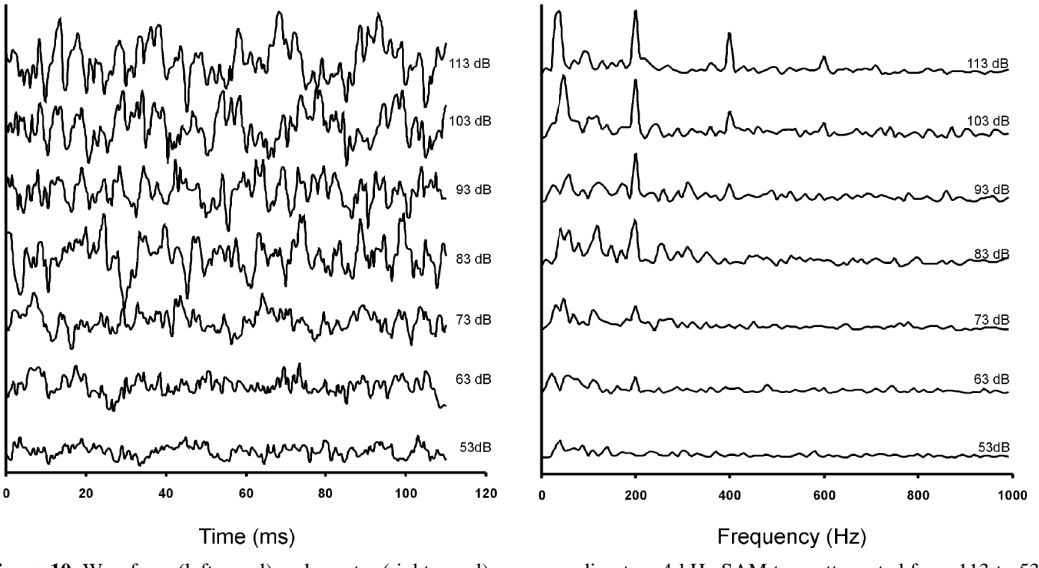

Figure 10. Waveform (left panel) and spectra (right panel) corresponding to a 4-kHz SAM tone attenuated from 113 to 53 dB; note the presence of the 2nd and 3rd harmonics of the amplitude modulation frequency (200 Hz) that are present at high stimulus levels.

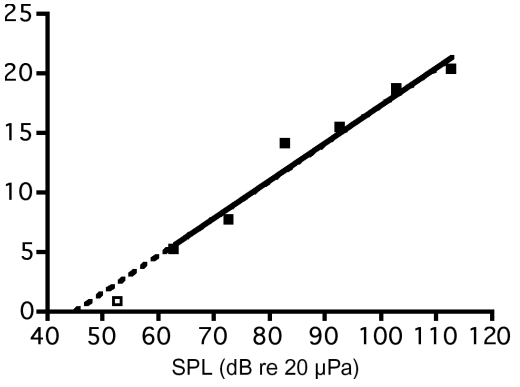

Figure 11. Change in the magnitude of the EFR with stimulus level for a 4-kHz SAM tone; filled symbols correspond to those signals which were objectively detected by application of the MSC technique, and open symbols indicate that no detection was made. The solid line results from the linear regression between the amplitude of detected responses and the stimulus level. The dotted line indicates the point where the threshold for 4 kHz is estimated by extrapolation to the 0 V crossing.

In this study, trains of rhythmic tone pips were used as stimuli to determine if this phenomenon could be capitalized on by looking at the repetition of evoked responses in the frequency domain. A comparison of 100-, 200-, 300-, and 400-Hz presentation rates demonstrated that waveform amplitudes and shape were most preserved at 100 Hz. The waveform quality of tone pip-evoked responses was diminished at presentation rates of 100 Hz, however, suggesting that recovery from prior stimulus presentation was not complete. Similar results are observed in the sea lion, which even at a 25-ms interstimulus interval does not show complete recovery for the specific response loci of the brain tested (Bullock et al., 1971). Nevertheless, in the elephant seal, the amplitude of the spectral peak that corresponds to the stimulus presentation rate scales with the amplitude of the stimulus. The degree of linearity of this relationship is dependent on both stimulus frequency and amplitude; the best linear response in spectral amplitude occurred using a 4-kHz stimulus at amplitudes < 65 dB re 20 µPa. Interestingly, the linearity of the relationship extended across a greater range of stimulus levels when the 2nd harmonic (200 Hz) was tracked; linearity was apparent at stimulus levels < 80 dB re 20 µPa.

The ability to track responses in the frequency domain prompted more investigation into the temporal resolving capability of the elephant seal as well as the potential for using more frequency specific techniques, such as the EFR, for estimating hearing sensitivity. Across the range of modulation rates tested, the MRTF of the elephant seal was maximal at 80 Hz. Phase angles demonstrate good linearity up to 800-Hz modulation rate. The linear relationship between the phase angle and modulation rate suggests a consistent source for generation of the potentials. The group delay calculated from the slope of the phase vs modulation rate function was 2.9 ms, which is less than group delays previously calculated for odontocetes across the same range of modulation frequencies (~4 ms) and slightly more than those for modulation frequencies above 2 kHz (~2 ms) (Supin & Popov, 1995).

Modulation frequencies at 80 Hz, though producing greater EFR amplitudes than higher modulation rates, require the high-pass filters of the AEP recording system to be lowered—in this instance, to 30 Hz. This results in the introduction of noise into the system which effectively reduces the signal-to-noise ratio of the AEP relative to those obtained with higher modulation rates. Therefore, the next most effective modulation rate, 200 Hz, was used in an attempt to track the EFR amplitude and estimate hearing sensitivity at 4 kHz (the high-pass filter was set to 100 Hz for these recordings). Extrapolation of the linear regression to the 0 V crossing resulted in a threshold estimate of 45 dB re 20 µPa. Aerial threshold values obtained outdoors with headphones for a trained elephant seal of similar age were found to be ~53 and ~44 dB re 20 µPa for 3.2 and 6.4 kHz, respectively (Kastak & Schusterman, 1998). In both the Año Nuevo tests (this study) and the tests of Kastak and Schusterman, it is likely that masking affected the threshold estimates. Recent work at LML in the hemi-anechoic chamber has demonstrated the behaviorally determined hearing sensitivity of the elephant seal at 3.2 and 6.4 kHz is lower than previously reported, most probably due to masking noise of the outside test environment (C. Reichmuth, unpub. data). Thus, the electrophysiological estimate of threshold seems reasonable; however, to date, no direct comparison of behavioral and EFR hearing thresholds has been made in an elephant seal. This process needs to be completed, as has been done for some odontocetes (Yuen et al., 2005; Finneran & Houser, 2006; Houser & Finneran, 2006), in order to quantify differences in thresholds predicted by the two methods and validate the approach for future use.

AEP methods currently applied to the study of odontocete hearing can also be applied to pinnipeds without the need for invasive, surgical procedures. Pinnipeds demonstrate a much attenuated neurophysiological response to acoustic stimuli relative to the odontocetes. The time required for testing is therefore necessarily lengthened because of the increased number of averages needed for high-quality recordings to distinguish the evoked

response from noise. These factors can be dealt with in wild and trained animals through the use of chemical immobilization or desensitization to the process via behavioral means. Tiletamine/zolazepam, ketamine, and diazepam are commonly used in the immobilization of elephant seals. The longitudinal study conducted here suggests that the use of dissociatives (tiletamine and ketamine) and zolazepam for immobilization has a negligible impact on the latencies or amplitudes of the short latency evoked responses studied here. Diazepam is commonly given for anxiety in humans and has been shown to have a mild effect on the latency of early evoked responses and no impact on the evoked response amplitude (Adams et al., 1985). The use of dissociatives and benzodiazepines (e.g., diazepam and zolazepam) is possibly as effective for studying auditory evoked responses in other phocids. In otariids, additional work needs to be conducted to determine the impact of anesthesia on evoked responses. Otariids may be immobilized with combinations of injectables as well as gas anesthesia (e.g., Haulena et al., 2000; Haulena & Gulland, 2001; Yamaya et al., 2006). To date, no studies have been conducted on otariids to determine how gas anesthesia affects responses evoked by acoustic stimuli.

Initial results of AEP studies conducted on the elephant seal hold promise for future, more in-depth characterizations of elephant seal auditory physiology. Furthermore, optimization of processes for estimating auditory sensitivity should advance knowledge of how AEP methods might be applied to larger, non-odontocete marine mammals. Methods that produced results in this study should be applied to larger age classes of elephant seals to determine how AEP measurements are impacted by increases in mass. The mass of adult male elephant seals may be in excess of 2,000 kg (Deutsch et al., 1994), more than 10 times the mass of animals between 1.3 to 1.8 y of age. Success at recording AEPs in these larger animals would be a step toward ultimately applying AEP techniques to other large mammals for which obtaining behavioral hearing data is difficult—possibly even mysticete whales.

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