Electrophysiological Assessment of Temporal Resolution in Pinnipeds

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Abstract

Studies of auditory temporal processing in marine mammals have traditionally focused on the highly refined temporal resolution capabilities of dolphins and other odontocete cetaceans. However, a recent electrophysiological investigation of manatee (*Trichechus manatus*) hearing has shown their temporal resolution to be better than expected, leading to speculation that enhanced temporal processing capabilities are adaptive for underwater sound localization. This study measured evoked responses from several California sea lions (*Zalophus californianus*), a harbor seal (*Phoca vitulina*), and a northern elephant seal (*Mirounga angustirostris*) to determine how well the auditory systems of these amphibious mammals resolve rhythmic stimuli. Trains of broadband clicks were presented in air at repetition rates from 125 to 1,500 s^{-1}, and the averaged evoked responses elicited by these stimuli were recorded from the skin. Rate-following responses were detected in the sea lions at rates up to 1,000 s^{-1}, with an upper limit of temporal resolution estimated at 875 to 1,000 s^{-1}. This upper limit is better than previously anticipated and was further substantiated by limited testing with the harbor seal and northern elephant seal. While these findings might support an underwater sound localization hypothesis, measurements comparable to those of the pinnipeds were also obtained in a phylogenetically similar terrestrial mammal: a domestic dog (*Canis familiaris*). It is therefore possible that increased temporal resolution in pinnipeds and other non-echolocating marine mammals is not a result of the evolutionary pressure of an aquatic environment.

Key Words: California sea lion, *Zalophus californianus*, harbor seal, *Phoca vitulina*, northern elephant seal, *Mirounga angustirostris*, pinniped, temporal resolution, evoked potential, electrophysiology

Introduction

Auditory temporal resolution plays a crucial role in the perception of complex sounds such as human speech (Stevens, 1983). Behavioral methods have been used to study the ability to detect changes in the temporal structure of acoustic signals in a variety of mammals (see Fay, 1988, for a review). While comparative psychoacoustic investigations of temporal resolution are optimal for describing how individuals of different species perceive the temporal features of sounds, these studies often require a great deal of time and training. A more efficient electrophysiological approach makes use of the auditory brainstem response (ABR).

One electrophysiological approach used to study auditory temporal processing is measurement of the rate following response (RFR) elicited by trains of repetitive clicks (see Supin et al., 2001). The RFR is composed of a series of ABRs that are generated in response to individual broadband clicks presented within a click train. When clicks are separated by relatively large temporal gaps, an individual ABR is generated in response to each click. As clicks become more closely spaced, individual ABRs begin to fuse into a sustained response that follows the repetition rate of the clicks. At lower repetition rates, the RFR is relatively high in amplitude, and it decreases in amplitude with increasing repetition rate. When the auditory system can no longer resolve individual clicks, there is only a response to the onset of the click train, which resembles an ABR elicited by a single click. The amplitude of the RFR as a function of click repetition rate resembles a low-pass filter shape, with the limit of the subject's temporal resolution typically defined as the upper cutoff of the amplitude-rate transfer function. The envelope-following response (EFR), which follows the modulation envelope of a sinusoidally amplitude-modulated (SAM) tone, has also been used to assess temporal resolution. Findings comparable to those of click train studies have been obtained using SAM tones, despite the

differences in spectral and temporal characteristics of the stimuli and the reduced amplitude of the EFR relative to the RFR (Supin & Popov, 1995; Popov & Supin, 1998).

Electrophysiological investigations of temporal resolution in marine mammals have been of particular interest due to the extraordinary auditory systems of dolphins and porpoises. Electrophysiological studies of RFRs in dolphins using rapid click train stimuli have revealed robust following responses to click repetition rates up to approximately 1,700 s^{-1} in the bottlenose dolphin (*Tursiops truncatus*) (Popov & Supin, 1998), 1,000 s^{-1} in the false killer whale (*Pseudorca crassidens*) (Dolphin, 1995) and Risso's dolphin (*Grampus griseus*) (Mooney et al., 2006), and 800 s^{-1} in the killer whale (*Orcinus orca*) (Szymanski et al., 1998). In comparison, electrophysiological studies of temporal resolution in terrestrial mammals have typically yielded lower limits of temporal resolution under similar experimental conditions (human [*Homo sapiens*] [Kuwada et al., 1986; Purcell et al., 2004], gerbil [*Meriones unguiculatus*] [Dolphin & Mountain, 1992], and chinchilla [*Chinchilla lanigera*] [Arnold & Burkard, 2002]). These findings have led to the hypothesis that rapid temporal processing in odontocetes is adaptive for echolocation and high-frequency hearing (Supin & Popov, 1995). Similar findings of extremely short temporal integration times in echolocating bats have further reinforced this hypothesis (e.g., Wiegrebe & Schmidt, 1996).

One group of marine mammals for which data on auditory temporal processing is lacking is the pinnipeds (seals, sea lions, and walruses). In terrestrial breeding colonies, vocal cues play an important role in a number of social contexts, including mother-pup attraction, male-male territory delineation, and mate attraction (e.g., Peterson & Bartholomew, 1969; Gisiner & Schusterman, 1991; Schusterman et al., 1992). Seals that breed underwater also rely on acoustic displays and songs for social interactions (see Tyack, 1999). Although pinnipeds do not echolocate as odontocetes do, their auditory systems play an important role in the passive detection of prey, predators, and conspecifics in marine environments in which other senses are limited (Schusterman et al., 2000). It is therefore probable that temporal resolution in the pinniped auditory system plays an important role in the processing of complex acoustic signals and, thus, individual fitness.

A few behavioral experiments addressing temporal integration of pure tones in the California sea lion (*Zalophus californianus*) and harbor seal (*Phoca vitulina*) (Terhune, 1988; Holt et al., 2004) have found that these subjects integrate tonal stimuli in a fashion similar to other mammals (see Fay, 1988). While these investigations do provide some insight into auditory temporal processing in pinnipeds, they are limited to pure tone stimuli and do not directly measure temporal resolution. Bullock et al. (1971) conducted a study of temporal resolution in the California sea lion by measuring responses from the midbrain evoked by trains of 0.5-ms clicks. Following responses were observed at click repetition rates of 300 to 800 s^{-1}. Unfortunately, the response magnitude as a function of click repetition rate is not described, making it difficult to estimate the low-pass cutoff of the amplitude-rate transfer function and to estimate an upper limit of temporal resolution.

A recent electrophysiological study of temporal resolution in another non-echolocating marine mammal, the Florida manatee (*Trichechus manatus*), documented maximal following responses to SAM tones at modulation rates of 150 and 600 Hz (Mann et al., 2005). This result places the temporal resolution abilities of the manatee in between those of examined terrestrial mammals and those of echolocating cetaceans. In accounting for the higher than expected temporal processing capability of the manatee, the authors suggest that increased ability to rapidly resolve the temporal structure of acoustic cues may be an advantage in localizing high-frequency underwater sound or in detecting the amplitude modulation within harmonic signals. In order to support such hypotheses, comparison of temporal resolution capabilities in mammalian auditory systems adapted for a fully or semi-aquatic lifestyle other than odontocete cetaceans is needed, along with data from phylogenetically related terrestrial species.

To address this goal, temporal auditory processing in California sea lions was examined in air using trains of repetitive broadband clicks. Furthermore, similar procedures were conducted with a Pacific harbor seal (*P. vitulina richardsii*) and a northern elephant seal (*Mirounga angustirostris*) to allow for comparison of results obtained using the same stimuli and identical electrophysiological procedures. As no appropriate data are available for referencing these results to those for a terrestrial carnivore, testing was also extended to a domestic dog (*Canis familiaris*).

Materials and Methods

General Procedure

Auditory evoked potentials (AEPs) elicited by trains of broadband clicks were obtained from apparently normal hearing individuals using subdermal electrodes arranged in a monopolar configuration. Click repetition rates were progressively increased in order to determine the relationship between response amplitude and

temporal patterning of stimuli. The experiment was completed with each subject during a single data collection session so that relative response amplitudes could be directly compared within subjects. At least one individual from each of the four species was tested in order to make interspecies comparisons. In the sea lions, four individuals were tested in order to gain a better idea of average temporal resolution capabilities and variability among subjects of the same species. Data collection took place between 12 May 2005 and 31 July 2006.

Subjects and Experimental Conditions
Sea Lions—The subjects were three male and two female California sea lions ranging in age from 1 to 4 y. The sea lions were identified as PDC, Wallaby, Amped, Tigerlilly, and Thistle. Testing took place at The Marine Mammal Center (TMMC) in Sausalito, California, where the sea lions were undergoing treatment following stranding and subsequent rescue along the California coast. The animals were tested under general anesthesia for medical procedures or prior to necessary euthanasia. The subjects were administered Telazol® (1.0 mg/kg) and metatomadine (0.04 mg/kg) through intramuscular injection prior to intubation, and gas anesthesia with isoflurane. Subjects were ventrally positioned on a surgical table and not handled during data collection. Overhead lights and unnecessary equipment were turned off during testing in order to reduce potential electrical noise contamination. A veterinarian attended to the sea lions during testing and monitored vital signs with a capnograph, esophageal ECG, and pulse oximeter. The equipment and experimenter were positioned a few meters from the animal during testing.

Harbor Seal—The subject was a 16-y-old male Pacific harbor seal identified as Sprouts who was permanently housed at Long Marine Laboratory at the University of California–Santa Cruz. Testing took place in a sound attenuating hemi-anechoic chamber (Eckel Industries). The experimenter and testing equipment were located in an adjacent, acoustically isolated control room. The subject was trained for voluntary participation in the experimental procedure and was awake and unmedicated during testing. Prior to the current experiment, the harbor seal had been conditioned using fish reinforcement to accept placement of the recording electrodes and to rest quietly in a fixed prone position during evoked potential data collection (for details, see Reichmuth et al., this issue).

Elephant Seal—The subject was a 1-y-old male northern elephant seal identified as SEP26. The seal had been collected from the wild at Año Nuevo State Reserve on the day of testing and transported to Long Marine Laboratory. Testing took place in the same acoustic chamber as used for the harbor seal. The elephant seal was given ketamine (0.5 cc) and diazepam (0.5 cc) intermittently by intravenous injection during testing as described by Houser et al. (this issue). During testing, the seal was positioned securely on a plastic restraint board where it rested quietly. The animal was not handled during electrophysiological measurements. The elephant seal was released within 24 h of capture at the location where it was collected.

Dog—The subject was a 6-mo-old male Golden Retriever identified as Bodhi. Testing occurred in an indoor room at Long Marine Laboratory. Prior to testing, the dog was administered acepromazine (0.05 mg/kg) by a veterinarian who was present throughout the experimental session. The dog rested or slept quietly on a mat during testing.

Acoustic Stimuli
For all subjects, stimuli were produced using trains of 133 μs biphasic rectangular pulses consisting of an initial upsweep followed by an identical inverted downsweep. These electrical pulses were generated using custom *LabView®* software installed on a laptop computer and sent through an NI DAQ-6062E card to a NI SCB-68 breakout box. The analog signals were then amplified using custom hardware and transmitted through a Morel MDT37 tweeter that was positioned approximately 0.5 m directly in front of the subject on the same horizontal plane. The duration of the acoustic pulse was approximately 1 ms (Figure 1A). The use of a remote speaker served to minimize stimulus artifacts that could obscure RFR analysis (Goldstein & Aldrich, 1999). The stimulus level for a single click measured at the position of the subject's ears ranged from 80 to 89 dB peak-to-peak equivalent sound pressure level (SPL); preliminary testing showed that this level was sufficient to induce relatively large ABRs while minimizing stimulus artifacts. The received click spectrum measured at the position of the subject's ears was similar to that of the frequency spectrum of the electrical pulse: peak energy is at approximately 3 kHz with a -25 dB cutoff at approximately 12 kHz (Figure 1B). A small peak existed above this with main energy between 16 and 22 kHz.

Click trains had durations of 20 to 24 ms in order to maintain a consistent onset of click stimulation. The click repetition rates presented during testing varied between 125 to 1,500 s^{-1}. Click trains were presented at rates of 11.7 s^{-1} to 14.7 s^{-1}, which were sufficient to preclude the potential confounding effects of long-term adaptation of the auditory system (see Popov & Supin, 1998).
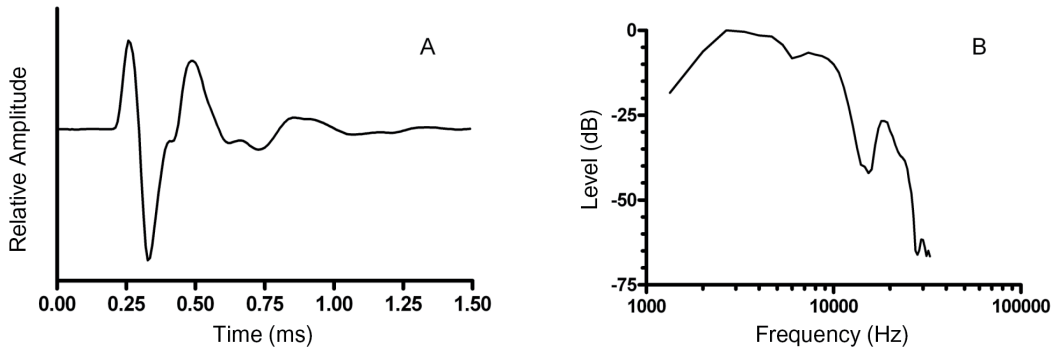
Figure 1. Waveform (A) and spectrum (B) of the click emitted from the speaker

Evoked Potential Recording

The AEPs were recorded from 12 mm × 30-gauge stainless steel subdermal needle electrodes (Grass F-E3M-72). Three sterile electrodes were inserted into the skin in a common reference configuration (a non-inverting cephalic electrode, an inverting noncephalic reference electrode, and a ground electrode). For the pinnipeds tested, the active electrode was placed on the caudo-rostral midline, 2 cm forward of the midline transecting the ears. This position measured the maximal ABR amplitude as determined by previous testing (Reichmuth et al., this issue). The ground and reference electrodes were placed dorsally behind the ribcage. The same electrode placement was used for the dog, which was consistent with reported data on response amplitude mapping in this species (Holliday & Te Selle, 1985).

The incoming electrophysiological signals were amplified 25,000 times with a customized biopotential amplifier, filtered, and then sent back to the computer through the same breakout box and data acquisition card. The responses of the first sea lion tested (PDC) were not filtered; however, all other records were passed through a Krohn-Hite Model 3530 filter with a high-pass setting (-3 dB) of 220 Hz and low-pass setting (-3 dB) of either 670 or 1,450 Hz (12 dB/oct rolloff outside of passband). Although a number of following responses were attenuated using these settings, response amplitudes were corrected *post hoc* for attenuation due to filtering. The number of stimulus sweeps averaged per trial ranged from 1,000 to 2,000. The sampling rate was 20 kHz for the sea lions, elephant seal, and dog, and 32 kHz for the harbor seal. The recording window was set to 40 ms for all trials in order to fit the stimulus presentation, the corresponding RFR, and at least 10 ms following the termination of the response.

Analysis

Frequency analysis of following responses was conducted for all subjects with filtered recordings. Fast Fourier transforms (FFTs) were conducted on a 16-ms window containing the RFR in all subjects. The FFTs contained 320 points for the sea lions, elephant seals, and dog, and 512 points for the harbor seal. The amplitudes of the observed RFRs were further evaluated for the four sea lions. First, average peak-to-peak amplitude measurements of individual waves within an RFR were calculated for each following response. In order to confirm these initial estimates of RFR peak-to-peak measurements, amplitudes were re-estimated for each following response by calculating the square root of the sum of the fundamental and harmonics from 125 to 1,500 Hz following Szymanski et al. (1998). Delay of the RFR was measured for sea lion PDC by taking the average delay from the end of a click train to the end of the RFR, and then correcting for the acoustic delay of the stimulus. PDC was the only subject for which the delay was reported as filtering of responses introduced phase lags that interfered with accurate latency measurements.

Results

ABR to Single Clicks

The ABRs measured in response to single clicks for all of the pinnipeds tested were similar in amplitude, wave delay, and composition to those described by Reichmuth et al. (this issue). The amplitude of the ABR in the four sea lions ranged from 770 nV to 1.2 µV. The amplitude of the ABR in the harbor seal and northern elephant seal were both smaller, at approximately 500 nV and 560 nV peak-to-peak, respectively. The dog ABR was similar to those of the pinnipeds in wave composition; however, the overall amplitude was significantly larger, approximately 2.25 µV peak-to-peak. The amplitude and waveform of the dog's ABR were

consistent with those reported for canines by other investigators (see Kawasaki & Inada, 1994).

Response to Rhythmic Click Trains
Sea Lions—Examples of the evoked responses elicited by trains of rhythmic clicks are shown for a California sea lion in Figure 2. Regardless of repetition rate, the waveforms show an initial "on" response that resembles an ABR to a single click, with a latency of approximately 6.6 ms to the peak of the dominant negative wave, after accounting for the delay due to speaker distance. At low click repetition rates of 125 to 187 s^{-1}, responses comprised individual ABRs that follow the rate of click presentation. At presentation rates of 250 to 500 s^{-1}, the RFR became a complex, quasi-sustained waveform created by the fusion of individual ABRs. At rates above 500 s^{-1}, the RFR developed into a sinusoidal wave of progressively decreasing amplitude relative to that of the initial ABR. The last visually identifiable RFR appeared at 1,000 s^{-1}. Click presentation rates above 1,000 s^{-1} produced only a single ABR, much like the response to a single click. The delay of the RFR relative to the stimulus presentation for all repetition rates was approximately 7 ms in the sea
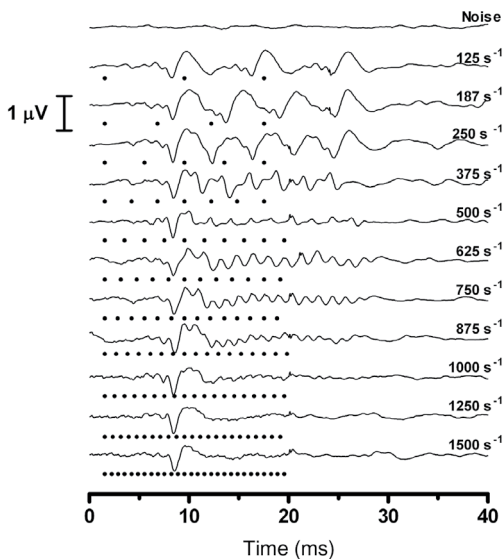
lion PDC as demonstrated by the persistence of the RFR waveform after the last pulse in a click train. This trend of consistent RFR delays across all click repetition rates is evident in Figure 2, although the delay appears slightly distorted due to phase lags introduced in filtering. This delay, along with the lack of a following response waveform before the initial onset response, suggests that the RFRs observed are indeed a physiological response and not an electromagnetic artifact of signal presentation.

The amplitude of the RFR as a function click repetition rate was determined for each of the four sea lion subjects by measuring the peak-to-peak amplitude of individual waves in the following response (Figure 3). All of the subjects showed a similar pattern of RFR amplitude, with the largest responses to click rates of 125 to 250 s^{-1}. Amplitudes decreased with increasing click rate past 250 s^{-1}, with the last visible response detected at 1,000 s^{-1} in all subjects.

The FFTs of the sea lion waveforms revealed the frequency composition of the following responses. The FFTs corresponding to the waveforms shown in Figure 2 are provided in Figure 4. Peaks at the frequency of click presentation rate are noticeable up to 1,000 s^{-1}. At 125 s^{-1}, peaks are apparent at multiple harmonics, with the peak at the first harmonic having larger amplitude than the peak at the fundamental frequency. At 187 s^{-1}, peak amplitude at the fundamental typically increased with respect to that at 125 s^{-1}. Peak amplitudes at harmonics of 187 Hz were less than that of the fundamental, a trend that continued as repetition rates increased above 187 s^{-1}. Fundamental peaks decreased with increasing click presentation rate past 187 s^{-1} until a very small fundamental peak remained at 1,000 clicks/s. Peaks were not well defined above 1,250 and 1,500 s^{-1}. In all four subjects, the dependence

Figure 2. Rate following responses (RFRs) to trains of rhythmic clicks in a California sea lion (Amped) for click rates of 125 to 1,500 s^{-1}; waveforms are averages of 2,000 evoked responses that were bandpass filtered from 220 to 1,450 Hz. Dots below waveforms indicate the onset of an individual click within a train, including the acoustic delay to the subject's head. RFRs are visually identifiable up to 1,000 s^{-1}. Note the persistence of the following response after the end of the stimulus train.
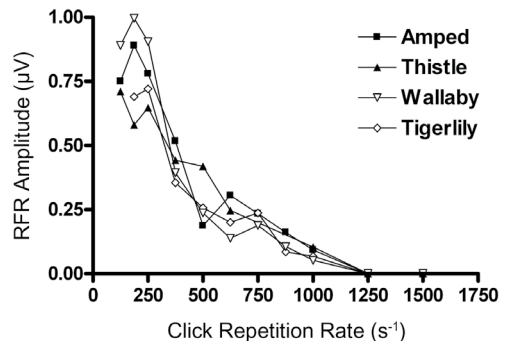

Figure 3. Amplitude of RFRs in four California sea lions; amplitudes were estimated by averaging the peak-to-peak amplitude of individual waves within a following response.
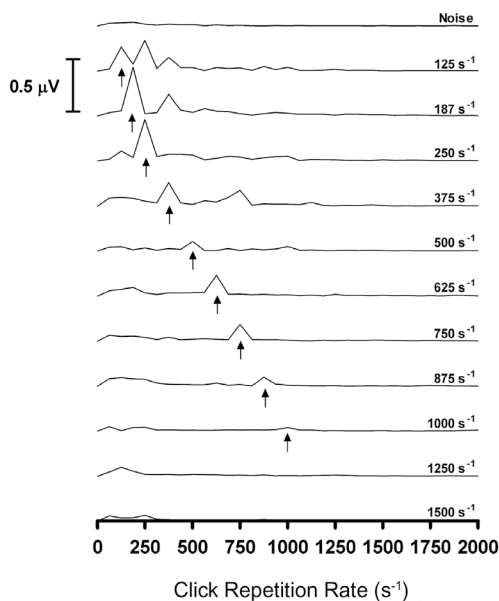
Figure 4. Fast Fourier transform of the RFRs shown in Figure 2; arrows indicate peaks at the fundamental of the click repetition rate. Note the presence of peaks at harmonics, especially at the low repetition rates.

of fundamental amplitude on click presentation rate (transfer function) had a low-pass filter shape, as depicted in Figure 5, with fundamental amplitude decaying to 10% (-20 dB) of its maximum amplitude at 875 to 1,000 s^{-1}. In most cases (three out of four subjects), there was a small peak in the transfer function at 750 s^{-1}. No peaks were visible at harmonics above 1,250 Hz in the FFTs of the RFRs. While there was no visible response at 1,250 and 1,500 s^{-1} in the recorded waveforms, amplitudes approximately 15 dB above the noise floor are visible at presentation rates of 1,250 and 1,500 s^{-1}. This can be attributed to the presence of a small artifact present in the FFT analysis window in these records. The artifact was most likely not a problem at lower presentation rates as the physiological response dominated the analysis window and was of a much larger amplitude than the artifacts in these records.

The amplitude of the sea lions' RFRs was re-estimated by computing the square root of the sum of the amplitudes at the fundamental and harmonics up to 1,500 Hz (Figure 6). In all subjects, RFR amplitude estimation was the highest at 125 s^{-1}, and it declined logarithmically with increasing click rate. Amplitude of the RFR with respect to click rate was similar in both estimation methods, with the exception of 125 s^{-1}. Rate following response amplitude at this rate computed using the second

method (Figure 6) was relatively large when compared to the measured amplitude using the first method (Figure 5). This is most likely due to the presence of harmonics that may not have been accounted for in the first method due to the phase relations of components in the RFR.

The general dependence of the RFR on click presentation rate displayed low variability among individuals as shown in Figures 5 and 6. Amplitudes of RFRs among subjects were similar, as were the harmonic composition of the RFR and the decay of the transfer function around 1,000 s^{-1}.

Harbor Seal and Elephant Seal—The records for the awake harbor seal and dissociated elephant seal were limited by fairly poor signal-to-noise ratios, making visual identification of transition points (i.e., individual ABRs, quasi-sustained, and sinusoidal) difficult. Results of FFTs of the RFRs, however, were similar to those of the anesthetized sea lions. Peaks at fundamentals were largest at the lowest click repetition rates with multiple peaks at higher harmonics. Fundamental amplitude dependence on click rate showed a similar pattern to that of the sea lions, with amplitude decreasing with increasing rate (Figure 7). For the harbor seal, there was a peak in the transfer function at 187 s^{-1}, a second peak at 625 s^{-1}, and a lower peak at 1,000 s^{-1}. The elephant seal transfer function also had maximum amplitude at 187 s^{-1}, a small peak at 750 s^{-1}, and a sharp drop-off past this point. Like the California sea lions, no harmonics were present above 1,250 Hz in the FFT of the seal RFRs.

Dog—The RFR amplitude as a function of click rate in the domestic dog displayed similar trends to those observed in all of the pinnipeds. Individual ABRs existed at low click presentation rates, became quasi-sustained at approximately 187 s^{-1}, and were sinusoid-like from 500 s^{-1} up to 1,000 s^{-1}. The RFRs of the dog were visually more apparent than in the pinnipeds due to the relatively
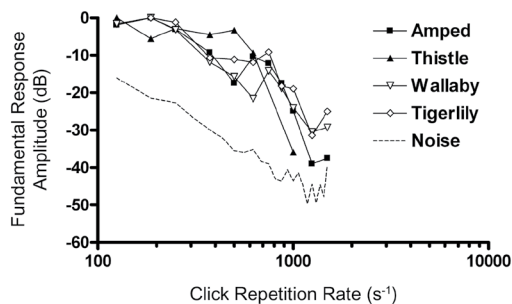

Figure 5. Amplitude of RFR fundamental as a function of click repetition rate in four California sea lions; amplitudes are shown as dB re maximum fundamental response for each subject. Averaged electrical noise obtained from stimulus absent trials in all subjects is shown for reference.
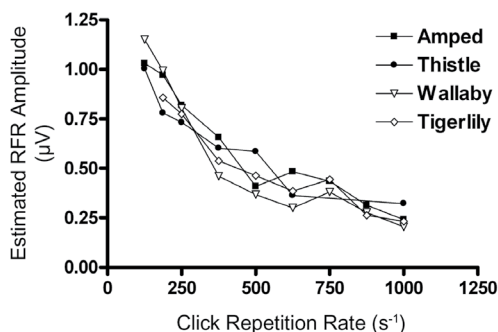
Figure 6. Amplitude of RFRs for four California sea lions when estimated as the square root of the sum of fundamental and harmonic amplitudes up to 1,500 Hz
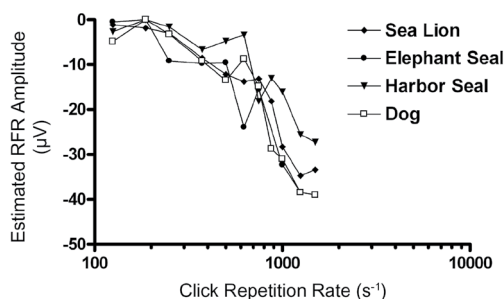

Figure 7. Amplitude of RFR fundamental as a function of click repetition rate in the California sea lion, northern elephant seal, Pacific harbor seal, and domestic dog. Data shown for the California sea lion are the average of the four subjects shown in Figure 5.

large amplitude of the ABR. Fast Fourier transforms of RFRs were similar to those from the pinnipeds, with multiple harmonic peaks present at low click presentation rates. The transfer function resembled a low-pass filter in shape, with a sharp drop-off in fundamental response amplitude after 625 s^{-1} (Figure 7). As with the sea lions, no peaks at harmonics were visible above 1,250 Hz in FFTs of the RFRs.

Discussion

The RFRs observed in this study appear to be generated by the fusion of multiple ABRs, a phenomenon that can be seen in the merging of single ABRs at low repetition rates into complex and sinusoidal waveforms at faster repetition rates. The dependence of the RFR fundamental on click rate corresponds roughly with the spectrum of the ABR in each of the subjects tested (see Reichmuth et al., this issue), although the relative amplitude of the fundamentals at higher click rates appears greater than the relative energy at corresponding

frequencies in the ABR spectrum. This can be explained by a property of the stimulus type used to evoke the RFRs. With trains of repeated clicks, the amplitude of the fundamental is proportional to the click presentation rate such that the fundamental at 1,000 s^{-1} is approximately 18 dB higher than that of a train of 125 s^{-1}. The ratio of the 1,000 Hz to 125 Hz response fundamentals is therefore higher than the corresponding frequency components in the response to a single click (Popov & Supin, 1998). This effect cannot be accounted for quantitatively in this study because the relationship between stimulus intensity and the amplitude of the RFR fundamental is unknown. The data are consistent with any predictions based on this property of the click train stimulus, however.

In the case of the sea lions, the calculated time lag of approximately 7 ms between the offset of the stimulus and the final wave of the RFR corresponds well to the delay of onset of the stimulus to the most prominent waves in the ABR. This further supports the notion that the RFR is generated by the fusion of successive ABRs, a phenomenon that has been observed in other RFR investigations (Popov & Supin, 1998; Szymanski et al., 1998; Mooney et al., 2006). Due to the consistent delay of the RFR across all repetition rates, it can be assumed that the RFR is most likely generated by structures in the sea lion midbrain at all of the tested click repetition rates (Supin & Popov, 1995).

The transfer functions of both fundamental amplitude versus click repetition rate and RFR amplitude versus click repetition rate both resemble a low-pass filter in shape for all of the individuals tested, much like the functions previously derived for other marine mammals using similar stimuli (see Supin et al., 2001). One distinct feature of the transfer functions available for odontocetes is the presence of peaks and troughs that correspond to presence or absence of frequencies in the ABR waveform. These peaks and troughs are not as noticeable in the sea lion data presented here, and this may be due to increased variability as RFRs in pinnipeds are of much smaller amplitude than those in odontocetes and therefore closer to the noise floor. Disregarding this, the transfer functions in this study resemble the ABR frequency spectra in that the highest response amplitudes are found below 275 s^{-1}, with diminishing amplitude as click rate increases. In defining an upper cutoff of temporal resolution from the amplitude-rate transfer functions, an arbitrary point can be chosen along the frequency axis based on the amplitude of the physiological response. Adopting the 10% (-20 dB) of maximum fundamental response amplitude level used by Popov & Supin (1998) as an upper limit of temporal resolution, it can be assumed the

California sea lions and the harbor seal have an upper limit of at least 750 to 1,000 s^{-1}, while the elephant seal's is at least 833 s^{-1}.

One concern with using the RFR to measure the upper limit of temporal resolution is the possibility that the following response is limited not by the actual ability of a subject's auditory system to follow repetitive stimuli but rather by the ability of the ABR to reproduce the frequency of click presentation. The ABR bandwidths of the pinnipeds tested contain a small amount of energy between 1,000 and 1,250 Hz (see Reichmuth et al., this issue), extending above the approximate limit of temporal resolution as defined above. Furthermore, the amplitudes of harmonics at low click presentation rates are of greater amplitude than the fundamentals at higher click presentation rates in both the sea lions and the harbor seal. It can therefore be assumed that the temporal resolution limits estimated in the pinnipeds are not limited by the bandwidth of the ABR.

It should be noted that a relatively robust fundamental response exists up to the region of 500 s^{-1} in the sea lions and the harbor seal, and to some extent, the elephant seal. This provides important information for the selection of stimuli suitable for EFR threshold audiometry. It has been demonstrated that the dependence of fundamental amplitude on click rate is similar to fundamental dependence on modulation rate of a SAM tone (see Supin et al., 2001), and if this trend holds true for pinnipeds, modulation rates above the region of significant ambient and extraneous biogenic noise but below the estimated limit of temporal resolution may prove useful in obtaining hearing thresholds to narrowband stimuli.

The availability of subjects in the present study allowed an assessment of intraspecific variability in temporal resolution to be made among the four sea lions tested. The close correspondence observed in response amplitudes as a function of click repetition rate suggests that auditory temporal processing capabilities vary little among apparently normal hearing individuals within a species. While only one elephant seal was tested in the current study, the results obtained correspond fairly well to an independent assessment of temporal resolution made in another elephant seal using similar stimuli (Houser et al., this issue). These findings lend additional weight to the limited data available for the two tested phocids as well as to most other studies of temporal processing in marine mammals that have sample sizes of only one or two individuals.

The data from this investigation suggest that temporal resolution in pinnipeds is relatively high in comparison to humans and other terrestrial mammals for which data are available.

The upper cutoff frequency of pinnipeds in this study, however, seems to be below the limits of temporal resolution of most odontocetes. Pinnipeds, therefore, appear to have temporal resolution capabilities that are similar to another mammal that made an evolutionary transition from a terrestrial to an aquatic lifestyle: the manatee. This correspondence is consistent with the hypothesis put forth by Mann et al. (2005) that evolutionary pressure in an aquatic environment has resulted in increased temporal resolution in non-echolocating marine mammals for the purpose of underwater hearing. This hypothesis is contradicted by the data obtained for the terrestrial carnivore (the domestic dog) in this study, however. The upper limit of temporal resolution in the dog is comparable to the investigated amphibious pinnipeds and the fully aquatic manatees, suggesting that evolutionary pressure for underwater hearing does not fully explain the increased temporal resolution capabilities of the non-echolocating marine mammals.

Properties of high-frequency peripheral filters in the auditory systems of non-echolocating marine mammals may partially account for the increased temporal resolution observed in electrophysiological studies. Temporal resolution is limited by the bandwidth of peripheral auditory filters, with narrow bandwidths optimal for frequency resolution and wide bandwidths optimal for temporal resolution (see Fay, 1992). Relatively wide filter bandwidths capable of high temporal resolution are found at the high-frequency region of the cochlea. Ultrasonic hearing has been demonstrated in the California sea lion, harbor seal, northern elephant seal, manatee, and dog (see Gerstein et al., 1999; Kastak & Schusterman, 1999; Dehnhardt, 2002), and it is conceivable that the high-frequency region of the cochlea plays a role in the increased temporal resolution capabilities demonstrated in these species. This hypothesis is supported by experiments demonstrating that high-frequency regions of the cochlea are important for temporal resolution in both humans and chinchillas (Fitzgibbons & Wightman, 1982; Fitzgibbons, 1983; Henderson et al., 1984).

A caveat to the comparison of results across investigations of temporal resolution lies within the variety of methods used in experiments. The use of different stimuli and recording techniques may yield variable results and, as a result, care must be taken in comparing data arising from disparate studies. The assessment of temporal resolution capabilities included in the present study attempted to avoid potentially confounding results by directly comparing data collected under similar conditions. Comparative electrophysiological investigations of temporal resolution need to

additionally consider the origin of a following response within the auditory nervous system. Studies that compare limits of temporal resolution derived from cortical electrophysiological responses (such as the commonly referenced 50 Hz EFR in humans) to those derived from ABRs in marine mammals compare two fundamentally different processes. For example, following responses of brainstem origin can be found at stimulus modulation rates up to 500 Hz in humans (Purcell et al., 2004). In comparative studies of temporal resolution using brainstem-derived following responses, reference to such values would provide for a more accurate comparison between species. Finally, investigators should avoid false interpretation of electrophysiological data as the perceptual experience of a subject. Although evoked potentials are a useful tool for studying the underlying physiological mechanisms governing auditory perception, psychophysical experiments provide the only means of determining the actual percept of a subject.

Acknowledgments

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