The binaural click-evoked auditory brainstem response of the California sea lion (*Zalophus californianus*)

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Auditory brainstem responses (ABRs) elicited by high-amplitude [100 dB re 20 μPa, peak-to-peak equivalent sound pressure level (peSPL)] aerial broadband clicks were collected from seven California sea lions in order to provide a basic description of short-latency auditory evoked potentials in this species. The waveform of the ABR was similar to that of other mammals, comprising seven positive and six negative characteristic waves. Variability in the amplitudes and latencies of waves was higher among subjects than the variability in within-subject repeated measurements. ABRs to progressively attenuated clicks were collected for three additional sea lions. Wave amplitudes decreased and latencies increased with decreasing stimulus level, with only the sixth positive wave visible near threshold (35–40 dB peSPL). Based on observations of wave latency as a function of stimulus amplitude, the sixth positive wave of the ABR is equivalent to the clinically important "wave V" identified in studies with humans. The current results provide information on the basic electrophysiology of the pinniped auditory system, including the processes that underlie brainstem auditory steady-state responses used to measure frequency-specific hearing sensitivity.
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I. INTRODUCTION

The mammalian auditory brainstem response (ABR) comprises electrophysiological potentials that generally occur within 10 ms following acoustic stimulation (Jewett and Williston, 1971; Huang, 1980). These potentials are seen as waves in the time-domain representation of the ABR, and are generated by structures in ascending auditory pathways, starting with the basal (high-frequency) end of the cochlea, and including the vestibulocochlear nerve, cochlear nucleus, olivary complex, lateral lemniscus, and inferior colliculus (see Hall, 2007). Reliable recording of the ABR is facilitated by its resistance to most anesthetic agents and the fact that it does not require the active participation of subjects (see Hall, 2007). As such, screening of auditory system health through ABR analysis has become a routine clinical procedure with humans, and it is useful in rapidly assessing some aspects of hearing in animals (Strain, 1992; Merzenich *et al*., 1983; Supin *et al*., 2001).

Due primarily to concerns regarding increasing levels of anthropogenic noise in marine environments (see, e.g., National Research Council, 1994, 2005; Southall *et al*., 2007), the assessment of marine mammal hearing capabilities through recording of brainstem responses has recently become particularly attractive for testing animals that are not trained for voluntary participation in psychophysical procedures. This is best demonstrated by studies of hearing sensitivity with a few marine mammal species that have measured brainstem responses like the ABR (Popov and Supin, 1990; Wolski *et al*., 2003, Houser *et al*., 2008), and brainstem auditory steady-state responses (ASSRs), which are generated by rhythmic ABR waves that are phase-locked to rapid modulation rates (e.g., >80 Hz in humans) imposed on an acoustic stimulus (Cohen *et al*., 1991; Kuwada *et al*., 1986; Lins *et al*., 1995). Several studies have examined cetacean hearing using brainstem ASSRs evoked at stimulus modulation rates as high as 1 kHz (Klishin *et al*., 2000; Houser and Finneran, 2006; Popov *et al*., 2007; Pacini *et al*., 2011; see Supin *et al*., 2001). Odontocete cetaceans are particularly suitable for this type of testing due to their refined temporal processing and the hypertrophy of neural structures from which ABRs and ASSRs of brainstem origin arise (see Supin *et al*., 2001). More recently, brainstem ASSRs have been used to non-invasively measure the aerial hearing sensitivity of sea lions (Mulsow and Reichmuth, 2010; Mulsow *et al*., 2011a, 2011b).

The amplitudes, latencies, and neural generators of the waves that comprise sea lion ABRs have not been thoroughly described. Some initial description of the auditory neurophysiology of the California sea lion (*Zalophus californianus*) was provided by Bullock *et al*. (1971). Evoked potentials were recorded from intracranial electrodes placed directly within the brainstem of both anesthetized and awake sea lions, allowing the authors to confirm the involvement of certain structures (e.g., the inferior colliculus) following auditory stimulation, as well as to explore some aspects of frequency-specific hearing sensitivity and auditory temporal processing. More recently, Reichmuth *et al*. (2007) qualitatively described some aspects of the California sea lion ABR recorded from non-invasive subcutaneous electrodes.

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Notably, relatively elevated ABR thresholds elicited by tone bursts for one older female sea lion in this study suggested age-related hearing loss. In studies with closely related Steller sea lions (*Eumetopias jubatus*, Family Otariidae), Mulsow (2009) also observed reduced amplitudes and increased latencies of ABR waves in a seven-year-old female relative to other tested Steller sea lions. Hearing thresholds determined with this female subject using ASSR methods were later found to be markedly elevated above those of other subjects (Mulsow, 2009; Mulsow *et al.*, 2011b). Aberrations in the sea lion ABR may therefore be indicative of hearing abnormalities, and their recording may be a rapid first step in hearing screening procedures for these marine mammals.

In an effort to produce a quantitative description of the California sea lion ABR, this study reports the waveform characteristics of ABRs evoked by broadband aerial clicks. Binaural click stimuli were used to elicit relatively high-amplitude ABRs from anesthetized sea lions in order to measure species-typical wave latencies and amplitudes. The variability in these features was determined, and ABRs to progressively attenuated clicks were recorded to provide a description of the ABR as a function of stimulus amplitude and to determine which features are homologous to those of ABRs recoded in other species. These measurements complement the previous basic descriptions of California sea lion ABRs made by Reichmuth *et al.* (2007) and support the further development of audiometric methods by providing information on the physiological processes of the otariid auditory brainstem.

II. MATERIALS AND METHODS

A. Subjects

The subjects of the study were ten California sea lions (nine female and one male) temporarily housed at The Marine Mammal Center in Sausalito, California. The disparity between males and females in the subject pool was due to the opportunistic nature of testing; the majority of California sea lions that stranded at The Marine Mammal Center during the study period were female. One of the female subjects, a juvenile identified as MBI, had a low body temperature in comparison to the other subjects, and is therefore treated independently in analyses of ABR waves (see below). Testing took place at the on-site surgical room while the subjects were under anesthesia prior to euthanasia. Euthanasia was the planned outcome for these subjects as they exhibited signs of non-recoverable domoic acid toxicosis following stranding events along the central California coast. Although domoic acid has neurotoxic effects at the level of the limbic system, it does not appear to cause damage to the brainstem (Silvagni *et al.*, 2005). During evoked potential recordings, the sea lions were maintained under gas anesthesia with 1%–2% isoflurane, following routine administration of butorphanol, midazolam, medetomidine, and in some cases, atropine. While under anesthesia, the subjects were monitored continuously by an attending veterinarian using a capnograph, esophageal ECG, pulse oximeter, and rectal temperature probe.

B. Acoustic stimulus and calibration

The stimuli used to evoke ABRs were dc square waves of 100 μs duration (*clicks* hereafter) presented at a rate of 17.1/s (Fig. 1). The polarity of the pulses alternated between positive and negative on successive presentations to reduce electrical artifacts in the electrophysiological records. The clicks were generated with an update rate of either 0.5 or 1 MHz using a laptop computer with Evoked Response Study Tool software (EVREST, Finneran, 2008, 2009), and a National Instruments USB-6251 data acquisition card. The clicks were passed through a Krohn-Hite 3 C series low-pass filter module (−3 dB at 150 kHz) followed by attenuation with custom hardware. The conditioned signals were then sent binaurally to a pair of Telephonics TDH-39 headphones that had been augmented with sound attenuating "earmuffs" that surrounded the TDH-39s and passively attenuated ambient noise without altering the natural orientation of the subjects' pinnae. As binaural presentation of stimuli elicits ABRs of higher amplitude than monaural presentation of stimuli with the same amplitude (van Olphen *et al.*, 1978), binaural stimulation was used to provide the highest possible signal-to-noise ratios.

The clicks were calibrated by placing an ER-7C probe microphone (0.25–14 kHz ± 3 dB) underneath the headphone within 2 cm of the base of the pinna. Signals received from the microphone were low-pass filtered using a second Krohn-Hite 3C series filter module (−3 dB at 200 kHz) followed by analog-to-digital conversion by the USB-6251 card and subsequent

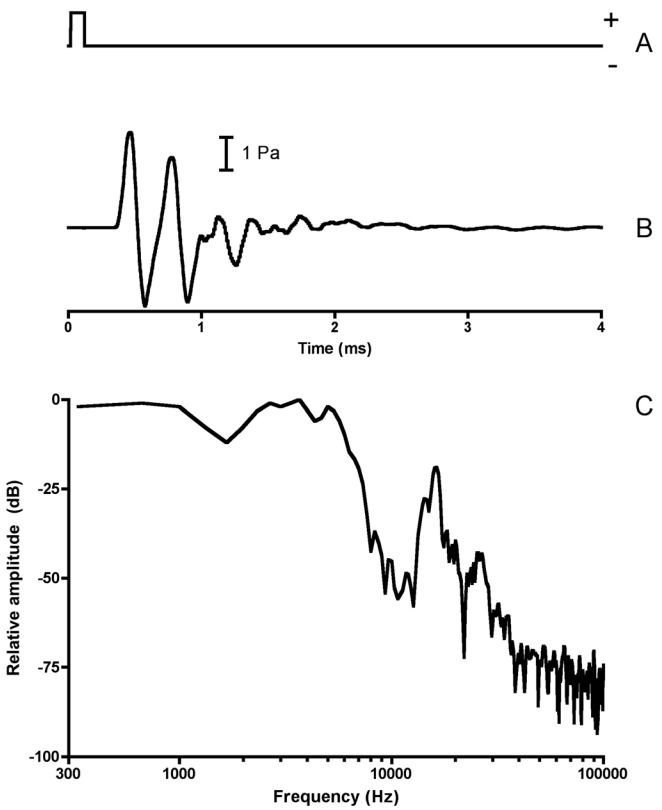

FIG. 1. The broadband click stimulus used to elicit California sea lion ABRs. (A) The 100-μs electrical pulse. The polarity of the pulse was alternated on successive epochs to reduce electrical artifacts in the recordings. (B) Representative waveform of the click recorded near the subjects' pinna. (C) Spectrum of the stimulus.

recording by EVREST with a sampling rate of 0.5 MHz. The stimulus level at the pinna was 100 dB re 20 μPa peak-to-peak equivalent sound pressure level (peSPL) for wave amplitude and latency measurements, and varied for click threshold measurements (see "ABR recording and analysis," below).

Ambient noise levels in the testing environment were measured underneath the headphones using the ER-7C probe microphone. The 1/3-octave band levels of noise ranged between approximately 20 and 40 dB re 20 μPa between 0.2 to 20 kHz. Actual received noise levels at the subjects' ears were lower than these levels, however, as the headphones attenuated ambient noise by 3–17 dB at frequencies above 1 kHz. Detailed noise levels in the testing environment are described in Mulsow *et al.* (2011b).

C. ABR recording and analysis

Subject ABRs were recorded using an electrode montage consisting of three 12 mm × 30 gauge stainless steel subdermal needle electrodes (Grass F-E3M-72). An active (non-inverting) electrode was placed on the dorsal midline of the head directly between the ears. A reference (inverting) electrode was placed on the dorsal midline, just posterior to the nape of the neck and near the scapula; this position had been previously determined to be relatively inactive with respect to the ABR in California sea lions (Reichmuth *et al.*, 2007). A ground electrode was placed just posterior to the ribcage. Incoming electrophysiological signals were amplified by 100 dB and band-pass filtered (0.03–3 kHz, −6 dB at cutoffs) using a Grass Technologies IP511 differential biopotential amplifier. Following amplification and filtering, electrophysiological signals underwent analog-to-digital conversion with the USB-6251 card. Digital waveforms were sampled, recorded, and averaged synchronously with signal presentation by the EVREST software at a sampling rate of 31.25 kHz. Each recording epoch was 58.34 ms in duration. Final waveforms were the average of 500 individual epochs [a number found to be suitable during previous testing with pinnipeds; Reichmuth *et al.* (2007)], and two final waveforms were acquired for each subject. Any recording window containing a voltage of absolute peak magnitude greater than 25 μV was considered to contain spurious artifacts and was not included in the waveform average. Less than 30 epochs were typically rejected from each 500-epoch average using these settings, although higher numbers of rejects (between 30 and 70) were sometimes observed. Collection of the two 500-epoch ABR recordings typically took 2 to 3 min.

Seven subjects were presented with the same 100-dB peSPL click to evoke ABRs. Wave amplitudes for positive waves were measured to the nearest 0.01 μV by manually placing cursors at the peak of a wave (defined as positivity at the vertex electrode) and the bottom of the trough immediately following it in the time domain waveform (which comprised the negative "peaks"). Amplitudes are therefore noted using a combination of positive and negative waves (i.e., P1/N1, P2/N2, etc.). An exception is P7, where a local minimum amplitude was used to calculate amplitudes as no seventh negative wave was regularly observed.

Wave latencies were measured to the nearest 0.01 ms by placing a cursor at a "shoulder" of a wave immediately following the peak (i.e., where the amplitude started to decline following positive waves and increase following negative waves). Latency and amplitude values were calculated for each subject by taking the mean of the measurements made on each of the two 500-epoch replicate traces. Latency values were not corrected for either electrical or acoustic transmission of the stimulus.

Click-evoked ABRs elicited by successively attenuated stimuli were subsequently recorded in order to determine auditory thresholds for three additional sea lions (identified as DAW, BUS, and TOR). The amplitude of clicks was initially set to 95 dB peSPL. These three subjects were not included in the previous part of the study, as hardware changes to the setup for the attenuation series had limited the maximum peSPL to 95 dB. Two 500-epoch records were collected, as described above, in 5 dB intervals until threshold (the stimulus level corresponding to the last visually detectable ABR) was reached. Recordings were also made at sub-threshold levels in order to confirm the absence of a response at stimulus sound pressure levels (SPLs) at least 5 dB below the last visible response. Click-evoked ABR threshold testing took between 20 and 50 min to complete.

III. RESULTS

The peaks of seven positive and six negative characteristic waves in the ABR were identified in the California sea lions (Fig. 2). These peaks occurred within 8 ms following presentation of the click stimulus. Each distinctive

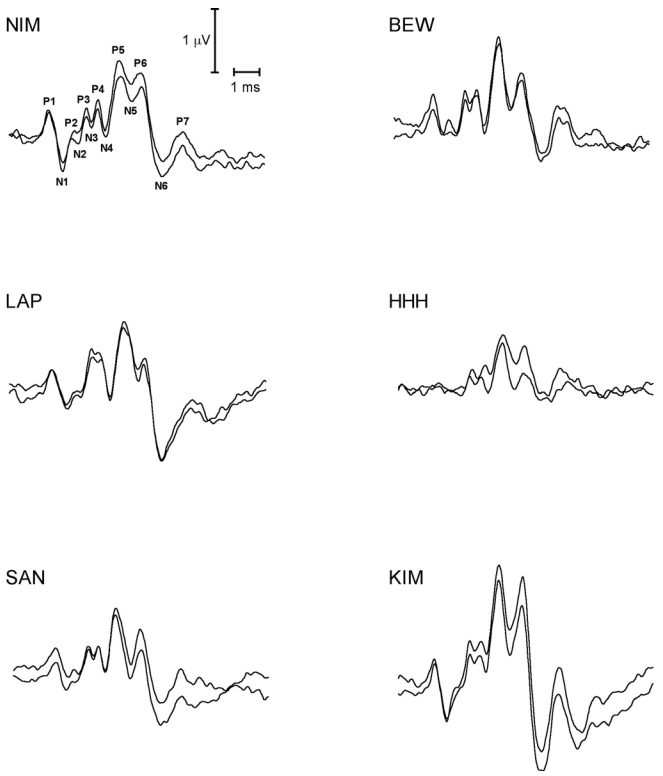

FIG. 2. The click-evoked ABR of six California sea lion subjects (the ABRs of subject MBI are not shown, see text). Each trace is the average of 500 individual recording epochs at the same stimulus intensity of 100 dB peak-to-peak equivalent SPL. Characteristic positive and negative waves are labeled using Arabic numerals. The beginning of each trace coincides with the onset of the click. Relative positivity at the vertex electrode is plotted upwards. The mean amplitudes and latencies of each individual's waves are given in Tables I–III.

TABLE I. Individual subject wave amplitudes (in μV) and mean values for characteristic waves comprising the binaural click-evoked ABRs of six California sea lions. The mean intra-subject amplitude difference (IAD) is the mean difference in amplitudes between the two 500-epoch records for each subject. Standard deviations are given in parentheses. Age classes of the subjects are given as S = sub-adult, A = adult.

Subject					Wave						
ID	Sex	Mass (kg)	Length (cm)	Age	P1/N1	P2/N2	P3/N3	P4/N4	P5/N5	P6/N6	P7
BEW	F	55	142	S	0.51	0.14	0.19	0.58	1.08	1.27	0.49
NIM	M	68	169	S	0.89	0.06	0.20	0.47	0.38	1.41	0.41
LAP	F	83	171	A	0.58	0.03	0.08	0.67	0.80	1.56	0.28
HHH	F	71	174	A	0.12	0.02	0.21	0.11	0.51	0.79	0.30
SAN	F	65	162	A	0.45	0.05	0.17	0.40	0.95	1.17	0.21
KIM	F	39	132	S	0.93	—	0.16	0.23	1.16	2.68	0.96
				Mean (SD)	0.58 (0.30)	0.06 (0.05)	0.17 (0.05)	0.41 (0.21)	0.81 (0.32)	1.48 (0.64)	0.44 (0.27)
				IAD (SD)	0.09 (0.09)	0.07 (0.06)	0.04 (0.05)	0.05 (0.01)	0.12 (0.09)	0.06 (0.05)	0.09 (0.06)

wave was labeled using an Arabic number system and a P or N for positive and negative, respectively. Waves P2 and N2, often appearing as a "shoulder" preceding P3, were not present in recordings for KIM. While P7 was present in all recordings, it was less "sharp" in terms of morphology than the other waves and did not have a clear negative wave following it. Although some subjects appeared to have additional waves in their ABR records, these waves were not identified with numeric labels, as they were not present in most subjects.

Mean amplitudes for the wave pairs (i.e., P1 max to N1 min) are given in Table I. Latencies for positive waves are given in Table II, and latencies for negative waves are given in Table III. The mean intra-subject differences for wave latencies and amplitudes between the two 500-epoch recordings for each individual were generally less than 0.01 ms and 0.01 μV, respectively. The degree of variability was larger among subjects than it was between the two 500-epoch traces acquired for each subject. The mean latency difference between P1, the first wave following the click, and the relatively large P6 for the six sea lions was 3.48 ms (SD = 0.12). The mean intra-subject difference in P1–P6 latencies for the two 500 average records was 0.03 ms (SD = 0.02).

The core body temperatures for these six sea lions was between 34 and 37.5 °C during testing. The juvenile sea lion identified as MBI (not included in Tables I and II) had an unusually low body temperature of between 33.5 and 34 °C during testing. The amplitudes of this subject's ABR waves had differences of +0.56, +0.10, +0.34, +0.12, −0.19, +0.39, and +0.06 μV relative to mean values (Table I) for waves P1/N1 through P7, respectively. Latencies of all but the first positive and negative waves of MBI's ABR were longer than the mean values for the other six sea lions. Latency differences were approximately −0.01, +0.38, +0.36, +0.49, +0.59, +0.64, and +1.29 ms relative to mean values for waves P1–P7, respectively, and +0.00, +0.41, +0.35, +0.40, +0.52, and +0.92 ms for N1–N6, respectively. The P1–P6 inter-wave latency for MBI was increased by 0.65 ms relative to other animals, a difference of more than two standard deviations.

The electrophysiological recordings from a click attenuation series for subject DAW, BUS, and TOR showed consistent changes in the ABR waveform with decreasing stimulus level. The recordings for subject BUS are shown in Fig. 3 for illustrative purposes. At the highest stimulus SPLs, the ABRs for DAW, BUS, and TOR were similar in terms of morphology to those of the other seven sea lions. As the amplitude of the click stimulus was attenuated, the amplitudes of the ABR waves decreased, and the latencies of waves increased. Response thresholds for the three subjects for which click attenuation series were conducted were 40, 40, and 35 dB peSPL. Wave P6 was the last wave detectable near threshold for all of the subjects. The mean latency of P6 for these individuals as a function of stimulus SPL is shown in Fig. 4.

TABLE II. Individual subject wave latencies (in ms) and mean values for characteristic positive waves comprising the binaural click-evoked ABRs of six California sea lions. The mean intra-subject latency difference (ILD) is the mean difference in amplitudes between the two 500-epoch records for each subject. Standard deviations are given in parentheses. Age classes of the subjects are given as S = sub-adult, A = adult.

Subject					Wave						
ID	Sex	Mass (kg)	Length (cm)	Age	P1	P2	P3	P4	P5	P6	P7
BEW	F	55	142	S	1.59	2.23	2.84	3.29	4.20	5.07	6.80
NIM	M	68	169	S	1.60	2.56	3.07	3.52	4.40	5.23	6.88
LAP	F	83	171	A	1.69	2.71	3.26	3.57	4.50	5.30	7.48
HHH	F	71	174	A	1.66	2.52	2.90	3.43	4.14	5.00	6.44
SAN	F	65	162	A	1.72	2.38	3.00	3.38	4.06	5.07	6.63
KIM	F	39	132	S	1.46	—	2.85	3.28	4.01	4.93	6.37
				Mean (SD)	1.62 (0.09)	2.48 (0.18)	2.99 (0.16)	3.41 (0.12)	4.22 (0.20)	5.10 (0.14)	6.77 (0.40)
				ILD (SD)	0.01 (0.01)	0.06 (0.03)	0.03 (0.03)	0.05 (0.03)	0.04 (0.03)	0.03 (0.01)	0.07 (0.03)

TABLE III. Individual subject wave latencies (in ms) and mean values for characteristic negative waves comprising the binaural click-evoked ABRs of six California sea lions. The mean intra-subject latency difference (ILD) is the mean difference in latencies between the two 500-epoch records for each subject. Standard deviations are given in parentheses. Age classes of the subjects are given as S = sub-adult, A = adult.

Subject					Wave					
ID	Sex	Mass (kg)	Length (cm)	Age	N1	N2	N3	N4	N5	N6
BEW	F	55	142	S	2.28	2.47	3.42	3.96	5.06	5.98
NIM	M	68	169	S	2.13	2.71	3.25	3.8	4.85	6.05
LAP	F	83	171	A	2.27	2.8	3.44	3.95	5.05	5.99
HHH	F	71	174	A	2.02	2.52	3.13	3.62	4.62	5.97
SAN	F	65	162	A	2.16	2.52	3.17	3.63	4.63	5.81
KIM	F	39	132	S	1.94	—	3.04	3.49	4.42	5.72
				Mean (SD)	2.13 (0.13)	2.60 (0.14)	3.24 (0.16)	3.74 (0.19)	4.77 (0.26)	5.92 (0.13)
				ILD (SD)	0.10 (0.10)	0.05 (0.06)	0.04 (0.04)	0.01 (0.02)	0.03 (0.04)	0.04 (0.03)

IV. DISCUSSION

The ABR of the California sea lion is similar to the ABR of other mammals in that it consists of approximately seven positive and six negative peaks occurring within the first 8 ms following acoustic stimulation (Huang, 1980; Merzenich *et al.*, 1983). As the neural generators of the California sea lion ABR are not yet confirmed, the Arabic labeling system (as opposed to the Roman numeral system) was used to avoid suggesting similarities in the sources of the California sea lion waves and those of the characteristic waves in other mammalian ABRs (e.g., Jewett and Williston, 1971). Nonetheless, it is reasonable to assume that the first few waves of the California sea lion ABR originate from relatively distal parts of the ascending auditory nervous system (such as the vestibulocochlear nerves and the cochlear nuclei) and the later waves from successively higher levels, a progression common to mammalian ABRs (Huang, 1980; Merzenich *et al.*, 1983; Hall, 2007).

Previous intra-cranially recorded potentials evoked by tone bursts provide some insight into the specific neural generators of the California sea lion ABR (Bullock *et al.*, 1971). Waveforms recorded from electrodes placed directly into sea lions' inferior colliculi feature a positive peak with a latency of approximately 5.4 ms, followed by a deep negative deflection. The general similarities in morphology and latency between this wave and the P6/N6 complex identified in this study suggest a contribution from the inferior colliculus to this portion of the California sea lion ABR. The inferior colliculus also contributes in part to the positive wave that immediately precedes a large negative trough in the ABR of other mammals including humans (e.g., Jewett and Williston, 1971; Hall, 2007) and dogs (Kawasaki and Inada, 1994): the clinically important wave V. Furthermore, P6 in the present study appears to increase in latency as well as persist longer than other waves of the ABR as stimulus

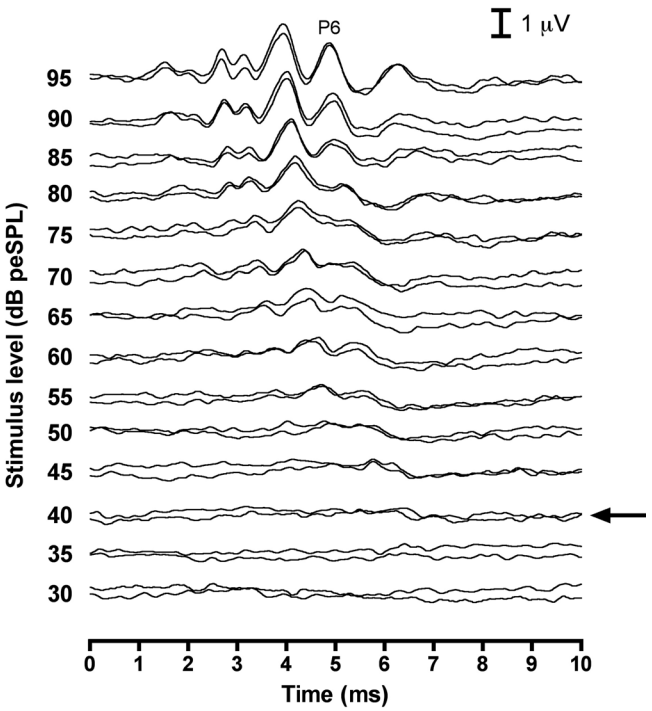

FIG. 3. Recordings of click-evoked ABRs in a sub-adult male California sea lion (BUS) at a variety of stimulus levels. Two records are shown for each stimulus level. Each record is the average of 500 individual recording epochs. At the highest levels, the characteristic waves of the ABR are clearly visible. Wave amplitudes decrease and latencies increase with decreasing stimulus level, until only P6 is visible. The arrow indicates threshold, defined as the last visually detectable response.

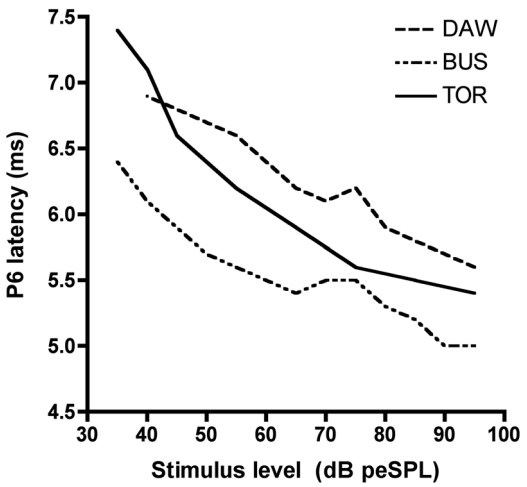

FIG. 4. Mean latency of ABR wave P6 as a function of stimulus level for three California sea lions. Measurements for each subject comprised the average of 500 epochs.

levels are decreased, both characteristic features of wave V. A homology of P6 from the California sea lion ABR and wave V in other mammalian studies can also be noted from previous ASSR studies. The brainstem ASSR in California sea lions in response to amplitude-modulated stimuli appears to be generated primarily by repetitive occurrences of the California sea lion P6 (Mulsow and Reichmuth, 2007). Likewise, human ASSRs at the level of the brainstem are thought to comprise repetitious occurrences of wave V (see Stapells *et al.*, 2005). Thus, P6 in this study seems to be equivalent to wave V described in studies with other mammals. Any further statements regarding the neural generators of the other waves comprising the California sea lion ABR are reserved for future studies.

Among the limitations of this study are the uses of binaural clicks to evoke ABRs, the alternation of the polarity of the clicks to avoid electrical artifacts in the records, and the testing of primarily female subjects. These factors are known to have diagnostic importance based on human studies (see Hall, 2007). Although binaural clicks result in higher signal-to-noise ratios relative to monaural clicks, ear-specific information is lost. Alternation of click polarity may obscure differences between ABRs to rarefaction and condensation clicks that may be present in high-frequency cochlear hearing loss, although these differences appear to have a large degree of inter-subject variability (e.g., Schoonhoven, 1992). Investigation of these topics in future sea lion ABR studies would be useful for establishing diagnostic baselines, as the signal-to-noise ratios found during this study are sufficient for monaural testing with unipolar clicks. A further limitation to these data is related to the opportunistic nature of testing; wave latencies and amplitudes are based only on sub-adult and adult subjects (as the juvenile MBI was excluded due to low body temperature), and only one male subject was able to be included. Age and sex-related differences in the ABR, such as reduced wave amplitudes with older human subjects and shorter latencies and larger amplitudes in female humans and rats (e.g., Beagley and Sheldrake, 1978; Church *et al.*, 1984; Jerger and Johnson, 1988; see Hall, 2007), are also relevant to the measurement of sea lion ABRs. These topics are potentially of interest to future research, especially considering the degree of sexual dimorphism present in California sea lions (Peterson and Bartholomew, 1967).

Changes in body temperature under gas anesthesia like those observed for one subject in this study have been shown to have an effect on the amplitude and latency of ABR waves in other mammals (Rossi and Britt, 1984). An increase in latencies (less than 0.5 ms) was found primarily for the later waves of the cat ABR as body temperature declined between 37 and 32 °C, and wave amplitudes increased with decreasing temperature to 32 °C, where values were on the order of 1 μV above normal. A similar trend of increased wave amplitudes, increased absolute wave latencies (especially for the later waves of the ABR), and increased P1–P6 latency was observed in the juvenile sea lion MBI, whose body temperature declined to between 33.5 and 34 °C during testing. Increases in ABR wave latencies with decreased temperature are likely due to delays in synaptic transmission and decreased axonal conduction velocity, with increases for longer latency waves being the most pronounced (Benita and Conde, 1972; deJesus *et al.*, 1973; see Hall, 2007). Increases in amplitudes with decreasing temperature are potentially related to reduced inhibitory activity from olivocochlear bundles terminating on cochlear hair cells (Galambos, 1956; see Hett *et al.*, 1995).

The ABR is fairly resistant to most anesthetic agents, and the effects of butorphanol, midazolam, and medetomidine in this study were most likely minimal (see Hall, 2007). However, Manninen *et al.* (1985) found increased latencies for the later waves (III, IV, and V) of the human ABR under isoflurane anesthesia, the largest of which was an approximately 0.66 ms increase for wave V for end-tidal isoflurane concentrations of 2%. Some additional variability in the amplitude and latency values reported for the California sea lions in this study may have therefore been introduced by small inter-subject differences in body temperatures or end-tidal isoflurane concentrations.

Nonetheless, the standard deviations for the latencies among subjects for all but two waves (P7 and N5) reported in this study are 0.20 ms or less, and the standard deviation of the P1–P6 relative latency difference was additionally small at 0.12 ms. There may be a slight increase in the latencies and amplitudes in our subjects relative to those of awake subjects due to isoflurane levels and decreased body temperature while under anesthesia. However, as ABRs recorded with sea lions under gas anesthesia provide high signal-to-noise ratios and do not require specific training of subjects (Reichmuth *et al.*, 2007), many future recordings of sea lion ABRs for hearing screening will likely be conducted with anesthetic and body temperature conditions similar to those in this study. Under such conditions, the wave latencies and amplitudes reported in the current study should be representative.

The lowest stimulus levels for which ABRs were visually detectable were similar for the three individuals included in this study. Although the sample size is limited, this suggests that these results are potentially representative for California sea lions tested under comparable conditions. In a previous study with California sea lions, Reichmuth *et al.* (2007) noted the last visually detectable click-evoked ABR at 49 dB peSPL with an adult male subject. Although this is elevated relative to the thresholds found in this study, the click stimulus used by Reichmuth *et al.* (2007) had different temporal and spectral characteristics. Also of note is the fact that Reichmuth *et al.* (2007) observed thresholds between 35 and 40 dB peSPL for a California sea lion using five-cycle tone bursts with center frequencies of 4 and 8 kHz. As is the case for human subjects, click-evoked ABRs in sea lions are likely the result of excitation at the basal (high-frequency) end of the cochlea. The similarity with the previous tone-burst thresholds may indicate activation in the 4 to 8 kHz region of the cochlea near threshold in the current click-evoked ABR data, as these frequencies are prominent in the click stimulus. However, predictions of frequency-specific hearing sensitivity based on click-evoked ABR thresholds alone are not supported by available data. Currently, the brainstem ASSR to modulated tonal stimuli, which the ABR underlies, is probably the best method of measuring tonal hearing thresholds using

electrophysiological methods in otariids (Mulsow and Reichmuth, 2010; Mulsow *et al*., 2011a; Mulsow *et al*., 2011b). A combination of ABR and ASSR methods, such as an initial observation of deviations from the wave amplitudes and the absolute and relative (i.e., P1–P6) latencies reported here, followed by measurement of frequency-specific aerial ASSR thresholds, is likely an efficient protocol for future sea lion hearing screening.

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