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Relating Click-Evoked Auditory Brainstem Response Waveforms to Hearing Loss in the Bottlenose Dolphin (*Tursiops truncatus*)

Krysta L. Gasser Rutledge,[1] Dorian S. Houser,[2] and James F. Finneran[3]

[1]*Washington University School of Medicine, Program in Audiology & Communication Sciences, 660 S. Euclid Avenue, Campus Box 8042, St. Louis, MO 63110, USA*
E-mail: gasserk@wustl.edu
[2]*National Marine Mammal Foundation (NMMF), 2240 Shelter Island Drive, Suite 200, San Diego, CA 92106, USA*
[3]*U.S. Navy Marine Mammal Program, Space and Naval Warfare Systems Center Pacific, Code 71510, 53560 Hull Street, San Diego, CA 92152, USA*

Abstract

Comparisons between click-evoked auditory brainstem responses (ABR) and auditory steady-state responses (ASSR) were performed to determine if the click-evoked ABR could be used to predict hearing loss in the bottlenose dolphin (*Tursiops truncatus*). The ASSR was evoked using sinusoidal amplitude modulated tones at half octave frequency intervals from 20 to 160 kHz and utilized to determine the upper-frequency limit of hearing in each dolphin (i.e., the frequency at which threshold was equal to 120 dB re 1 µPa). The click-evoked ABR was then recorded following exposure to a moderate-amplitude click (peak-peak equivalent sound pressure level of 122 dB re 1 µPa, 5 to 100 µs duration) and examined to determine if relationships existed among the upper-frequency limit of hearing and the amplitude/latency characteristics of the click-evoked ABR. The ASSR and click-evoked ABR were measured in six dolphins (4 males and 2 females, from 13 to 49 y of age) with varying hearing sensitivity and frequency range of hearing. A significant relationship existed between click-evoked ABR wave amplitudes and the upper-frequency limit of hearing, although the number of waves showing the relationship varied with the duration of the click. Test times for assessment using frequency-specific ASSR and click-evoked ABR were ~45 min and 1 min, respectively. With further definition of normative data, measurement of click-evoked ABRs could form the basis of an expedited electrophysiologic method for hearing screening in delphinids.

Key Words: odontocete, bottlenose dolphin, *Tursiops truncatus*, hearing assessment, auditory steady-state response, auditory brainstem response, presbycusis

Introduction

Auditory evoked potentials (AEPs) are small changes in voltage representing neural synchrony within the auditory nervous system in response to acoustic stimuli. The auditory brainstem response (ABR) is an AEP which has been generated specifically from the auditory nerve and within the auditory brainstem. In odontocetes, the ABR to a click or tone-burst stimulus is a robust and replicable response quantified by amplitude and latency values of seven waveforms, all occurring within about 6 ms of the stimulus onset (Bullock et al., 1968; Ridgway, 1980). As stimulus intensity decreases, the ABR waveform amplitudes decrease and latency values increase. Although tone-burst ABRs may be reliably obtained in odontocetes (toothed whales), most estimates of frequency-specific thresholds have utilized the auditory steady-state response (ASSR; also termed the envelope-following response or EFR). The ASSR is formed when stimuli are presented at a sufficient rate so that transient AEPs overlap and form a steady-state response (Galambos et al., 1981; Stapells et al., 1984). A common ASSR methodology involves presentation of sinusoidal amplitude-modulated (SAM) tones. The recorded neurophysiologic response follows the "envelope" of the amplitude-modulated carrier signal such that the ASSR is detected as a voltage peak at the modulation frequency—that is, the auditory neurons respond to the carrier tone but fire at the modulation rate (Galambos et al., 1981; Picton et al., 2003). In the bottlenose dolphin (*Tursiops truncatus*), peak amplitudes are recorded when using modulation frequencies ranging from 550 to 600 Hz and 1,000 to 2,000 Hz for high-frequency carrier signals (Dolphin et al., 1995; Supin & Popov, 1995; Finneran et al., 2007). The ASSR has shown good agreement with behavioral

measures of hearing sensitivity, although it typically underestimates behavioral sensitivity to some degree (Nachtigall et al., 2004; Houser & Finneran, 2006b; Finneran et al., 2008; Yuen et al., 2005). Additionally, more specific comparisons have established good agreement between electrophysiological (ASSR) and behavioral thresholds using a jawphone transducer placed on the pan region of the mandible (e.g., underwater ASSR thresholds vs underwater behavioral thresholds [Houser & Finneran, 2006a], aerial ASSR thresholds vs underwater behavioral thresholds [Finneran & Houser, 2006], and aerial ASSR thresholds and behavioral thresholds estimated from data collected simultaneously [Schlundt et al., 2007]).

Due to the potential negative impacts of anthropogenic noise upon marine mammals (see Miller et al., 2000; Holt et al., 2009), the National Research Council (NRC) has repeatedly documented the need for additional research required to better understand marine mammal hearing sensitivities and the physiological impact of sound on marine mammals (e.g., temporary threshold shift) (NRC, 1994, 2000, 2003, 2005). Recommendations such as establishing baseline hearing sensitivities in greater numbers of species and individuals representing these species have been outlined, which would benefit from AEP equipment that is hardy and portable (such as the system described in Finneran, 2009) and methodologies that are easily programmable for automaticity and time-efficiency, particularly in the case of field testing (i.e., stranded animals). In addition, the performance of hearing assessments in stranded odontocetes prior to a determination of whether the individual can be released following rehabilitation are becoming increasingly common. These assessments are critical to determining whether sufficient hearing exists to support echolocation, which is essential to odontocete foraging and navigation in the ocean environment.

The goal of the present study was to determine if ABRs generated in response to a suprathreshold click stimulus could be used to estimate the upper-frequency limit of hearing in *Tursiops truncatus*. Establishing a relationship between suprathreshold click-evoked ABR properties and the upper-frequency limit of hearing could provide a more expeditious methodology for hearing screening compared to ASSR threshold measurements performed at multiple frequencies, which is now commonly used to test odontocete hearing. The results of this study have potential applications to marine mammals in the wild, in rehabilitation (i.e., following stranding), and under long-term human care, particularly for the rapid determination of the presence of hearing deficits.

Methods

Subjects

Study subjects were Atlantic bottlenose dolphins in the care of the U.S. Navy Marine Mammal Program at the Space and Naval Warfare Systems Center (SSC) Pacific located in San Diego, California. Subjects included two dolphins with normal hearing frequency range (1 male, 1 female) and four dolphins with high-frequency hearing loss (3 males, 1 female), ranging in age from 13 to 49 y (Table 1). Hearing loss was defined in this study as an upper-frequency limit of hearing ≤ 120 kHz. All protocols were approved by the Institutional Animal Care and Use Committee of the Biosciences Division, SSC Pacific and the Navy Bureau of Medicine and Surgery, and followed all applicable U.S. Department of Defense guidelines for the care of laboratory animals.

Stimulus Presentation and Evoked Response Recording

All subjects were tested in floating, netted enclosures in San Diego Bay. During AEP measurements, subjects voluntarily submerged and positioned themselves on a "biteplate" with their dorsal surface above the waterline, allowing for respiration throughout the test sessions. Acoustic stimuli were presented to the subject utilizing a jawphone transducer (piezoelectric sound projector [Reson TC 4013] embedded in a V-1065 silicon rubber suction cup) placed on the pan

Table 1. Subject demographics

Animal ID	Gender	Age (y)	Weight (kg)	Upper-frequency limit of hearing (kHz)
COL	Male	13	197.7	**68.4**
TRO	Male	22	181.8	137.7
OLY	Male	30	190.4	**50.3**
TYH	Male	33	188.2	**82.8**
SAY	Female	35	244.4	128.1
BLU	Female	49	210.0	**48.5**

region of the left mandible (Moore et al., 1995; Brill et al., 2001). The jawphone transducer was calibrated with the same stimuli used for the study (SAM tones and clicks) at a distance of 15 cm from the transducer. This distance was used as it corresponds to the distance between the attachment point of the transducer on the lower jaw and the auditory bulla (Houser et al., 2004). Animals were rewarded with fish for remaining on the biteplate for the duration of the tests.

SAM Tone-Evoked ASSR—SAM tones generated by a portable auditory-evoked potential system (*EVREST*, detailed in Finneran, 2008, 2009; Finneran et al., 2009) were used to evoke an ASSR. The SAM tones consisted of one of seven carrier frequencies spaced at half octave steps from 20 to 160 kHz. Each SAM tone was 100% amplitude modulated at a rate of 1 kHz; this modulation depth and rate has been shown to be optimal for evoking a robust ASSR in the Atlantic bottlenose dolphin (Dolphin et al., 1995; Supin & Popov, 1995, 2000). All SAM tone stimuli were generated with a 1 ms rise/fall time and were 22 ms in duration.

Click-Evoked ABR—Click stimuli of various durations (5, 50, and 100 μs) were generated by transmitting a 1 V rectangular wave to the jawphone transducer using the *EVREST* system. The transmitted click had a peak-peak equivalent sound pressure level (ppeSPL) of 122 dB re 1 μPa (hereafter denoted as "dB SPL"). Clicks were presented to the dolphins at a rate of 46.8 clicks/s, and the polarity of the click was alternated on each presentation to cancel any potential artifacts from the stimulus presentation.

Evoked Response Recording—The ASSR was measured utilizing 10-mm gold-cup electrodes (Viasys Healthcare) embedded in 25-mm diameter silicon suction cups coupled to the skin using conductive paste. Electrodes were placed immediately prior to each test session in the following montage: noninverting (+) electrode ~10 cm posterior to the inferior margin of the blowhole and ~2 cm contralateral of the ear being tested; common (ground) electrode on the subject's back ~8 cm anterior of the dorsal fin; and inverting (-) electrode placed on the subject's back midway between the noninverting and ground electrodes (Popov & Supin, 1990) (Figure 1). Electrode signals were differentially amplified (100,000 gain), filtered (300 Hz to 3 kHz), and digitized at ~11.1 kHz for ASSR measurements and at 40 kHz for click-evoked AEPs. The signal rejection level (i.e., artifact rejection) was set at the beginning of each session based on the background electrophysiological noise observed prior to the beginning of sample collection.

Figure 1. Experimental setup for the collection of click-evoked and sinusoidal amplitude-modulated (SAM) tone-evoked potentials in bottlenose dolphins (*Tursiops truncatus*)

A magnitude-squared coherence (MSC) test was applied after 256 epochs (specified time period during which analysis occurs) to determine if the amplitude of the evoked response at the modulation frequency was significantly greater than measurement noise (Dobie, 1993; Dobie & Wilson, 1989, 1996). The test was repeated utilizing the cumulative number of epochs recorded every 256 epochs until the signal was detected or until a maximum of 1,024 epochs was recorded. Utilizing the ASSR that corresponded to full amplitude modulation of the stimulus (i.e., ignoring the rise/fall component), the MSC was calculated by dividing the total number of epochs obtained for each frequency/stimulus pairing into 16 subaverages. The MSC_{crit} for each test was obtained from Amos & Koopmans (1963) and Brillinger (1978) with $\alpha = 0.01$. Signals with a $MSC > MSC_{crit}$ were considered statistically different from noise and, thus, detected responses.

An automated modified staircase technique was used to adjust the stimulus SPL and record responses sufficient for threshold estimation. Data collection began with a stimulus level of 110 dB SPL (exception: testing at 160 kHz which began at 120 dB SPL). If a signal was detected, the SPL was reduced for the subsequent test. The initial change in SPL for subsequent tests began at 30 dB step size (exception: testing at 160 kHz which began at 10 dB). If the ASSR was not detected, the SPL was increased on subsequent tests until it was once again detected. The change in the step size on subsequent tests was adjusted upon each reversal; the step size was decremented by 0.45 of the prior step size when reversing from a nondetection to a detection, and was decremented by 0.40 of the prior step size when reversing from a detection to a nondetection. The testing concluded when the step size was ≤ 3 dB, and the threshold

was calculated as the difference between the lowest stimulus SPL producing a detectable ASSR and the highest stimulus SPL at which no ASSR was detected. Threshold testing was terminated if no detections were obtained with stimulus SPL ≥ 120 dB SPL. The upper-frequency limit of hearing was defined as the frequency at which the threshold was equal to 120 dB re 1 µPa. The frequency limit was determined by linearly interpolating between two frequencies with thresholds above and below the 120 dB criterion.

An ASSR-derived input/output (I/O) function was determined for each animal at each frequency for which a threshold < 120 dB SPL could be determined. To create the I/O function, the amplitude of the ASSR was first determined for a SAM tone stimulus of 40 dB sensation level (SL) (i.e., 40 dB above the initially determined threshold). (When thresholds were determined but stimulation at 40 dB SL was not possible, stimulation began at the highest stimulus level producible by the transducer without producing stimulus artifacts.) The stimulus SPL was decreased in 5 dB increments until 10 dB below threshold, and 1,024 epochs were recorded at each stimulus level tested. The amplitude of the evoked response spectra at the modulation rate, determined from the average of the 1,024 epochs, was subsequently plotted for each stimulus level presentation to determine the I/O function. If visual inspection of the data suggested a break-point within the I/O function (i.e., a notable change in the slope of the I/O function within the range of tested SPLs, a segmented regression analysis was used to determine if a break-point truly existed and whether two regression lines better characterized the nonlinearity of the I/O function.

The segmented regression compared the summed squared error of two regressions describing the distribution of the data with that of a single regression line across all data points. Data points for the segmented regression were constrained to consecutively ordered groups of data points. If any combination of consecutively grouped data points comprising the regression segments resulted in a lower summed squared error than the single linear regression, the segmented regression analysis was used to define the I/O function. If the I/O function was best fit with a single regression line, this was referred to as a *nonsegmented* I/O function. If the I/O function was best fit with two segmented regression lines, the two regression lines were referred to as the *low-frequency segment* and the *high-frequency segment*. Note that the terms of low and high frequency do not refer to specific frequencies but only the relationship of the two segmented regression lines to one another. Linear mixed models were utilized to see if the presence of hearing loss and the frequency tested affected the I/O function slopes or the presence of break-points. For mixed models, the subject was included as a random effect.

Procedures utilized for click-evoked ABR recordings were the same as those discussed for the ASSR above unless otherwise detailed. Six recordings of 1,024 epochs were collected in each animal and for each click duration (5, 50, and 100 µs). The 1,024 epochs were averaged to produce a grand average click-evoked ABR waveform, which was subsequently used for peak latency and amplitude measurements. Latencies and amplitudes (P1, N2, P3, P4, and N5) and interpeak latencies (P1-P4, N2-N5, P3-P4) were recorded for each subject at each click duration (5, 50, and 100 µs) by inspection of the ABR waveform. Linear mixed models and linear regressions were utilized to determine if any relationships existed among age and the upper-frequency limit of hearing (independent variables) and the peak absolute latencies, interpeak latencies, and wave amplitudes.

Results

Animal Description

Upper-frequency limits of hearing ranged from 48.5 to 138 kHz, with four animals exhibiting high-frequency hearing loss when compared to the expected range of hearing in a bottlenose dolphin (bolded, Table 1).

Click Spectra Analysis and Click-Evoked ABRs

The spectra for the 5 µs click ranged from ~20 to 150 kHz (-10 dB point criterion) with peak energy at ~125 kHz and a -10 dB bandwidth of ~57 kHz (~70 to 127 kHz; Figure 2). A peak in the spectra was also prominent around 55 kHz. A rippling effect was noted as click duration increased from 5 to 100 µs, with notches in the spectrum appearing at intervals corresponding to the frequency of the first null in the click spectrum (i.e., 10 kHz "ripples" for the 100 µs click).

The click-evoked ABR included five primary components recorded within the first 6 ms of the stimulus onset: P1, N2, P3, P4, and N5 with "P" indicating a positive deflection and "N" indicating a negative deflection (Figure 3). Figure 4 presents ABR recordings produced in response to the 5 µs click (122 dB ppeSPL) for each animal, ordered by descending upper-frequency limit of hearing. Average ABR amplitudes, latencies, and interpeak latencies are listed by animal and waveform component for each click duration in Tables 2 & 3. N5 was the dominant wave across all animals, whereas P3 was generally the dominant positive wave. Waveform amplitudes generally decreased and latencies increased as the upper-frequency limit of hearing decreased. However, there were

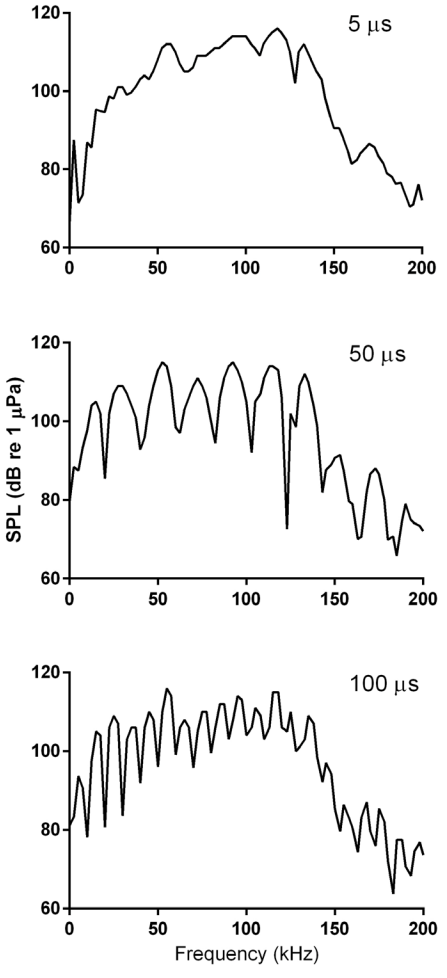

Figure 2. Frequency spectra corresponding to the 5, 50, and 100 µs clicks used in the click-evoked auditory brainstem response (ABR)

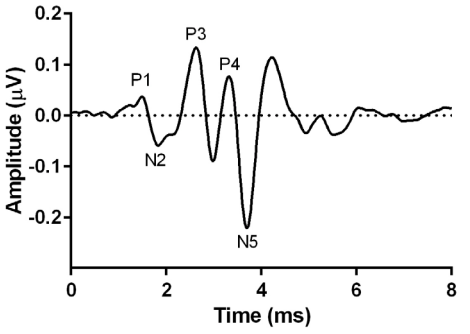

Figure 3. Waveform components (P1, N2, P3, P4, and N5) of an ABR produced in response to a moderate-intensity (122 dB SPL re 1 µPa) click of 5 µs duration for subject TRO.

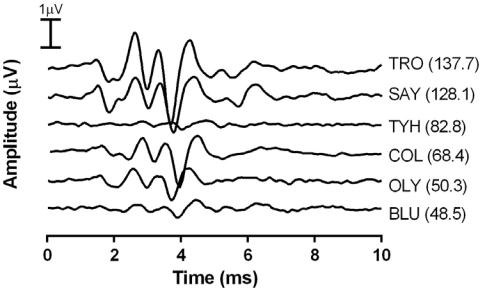

Figure 4. Click-evoked ABR waveforms for each bottlenose dolphin in descending order by each subject's upper-frequency limit of hearing (given in kHz and shown in parentheses beside the animal identifier); the ABR was produced in response to a 122 dB ppeSPL click of 5 µs duration.

individual differences that cannot be accounted for by the upper-frequency limit of hearing alone, (e.g., TYH had the lowest amplitude waves and OLY demonstrated shorter latencies than COL).

There was a significant positive relationship between the upper-frequency limit of hearing and the amplitude of all waves when considering the ABR to the 5 µs click ($r^2 = 0.73$ to 0.81; $p \leq 0.029$, $\alpha = 0.05$; Figure 5). However, at 50 and 100 µs, this relationship was only maintained for waves P1, N2, and P4 ($r^2 = 0.66$ to 0.74; $p < 0.049$, $\alpha = 0.05$). The relationship was not significant for waves P3 and N5 when produced with the 50 and 100 µs clicks, although the relationship trended in this direction (p values ranged from 0.07 to 0.09). No relationship among either click-evoked ABR waveform amplitude or latency and age, gender, or animal mass was noted.

SAM Tone-Evoked ASSR I/O Functions

Slopes of the *low-frequency segment* and nonsegmented I/O functions ranged from 0.5 to 3.5 nV/dB. The frequency tested significantly affected the slope of the low-frequency segment and the nonsegmented I/O function when animal ID was included as a random effect ($p = 0.04$, $\alpha = 0.05$). Whether or not an animal had hearing loss and the upper-frequency limit of hearing appeared to have no effect on the slopes of the low-frequency segments or nonsegmented I/O functions. Slopes of the *high-frequency segment* I/O function (following break-point), if present, were always steeper than the low-frequency segment or nonsegmented slopes and ranged from 6 to 68 nV/dB SPL. When a break-point was found, it always corresponded

Table 2. *Click-evoked ABR:* Waveform amplitude values

5 µs					
	Amplitude (nV)				
Animal	P1	N2	P3	P4	N5
COL	55.2	164	341	268	839
TRO	344	536	1,330	747	2,180
OLY	127	187	362	186	644
TYH	32.2	71.9	88.0	78.3	172
SAY	394	770	785	556	1,600
BLU	26.4	55.2	80.1	74.7	131
50 µs					
	Amplitude (nV)				
Animal	P1	N2	P3	P4	N5
COL	125	175	382	287	948
TRO	317	462	1,210	649	2,010
OLY	171	233	482	192	814
TYH	39.7	61.7	84.2	63.6	202
SAY	342	633	667	452	1,300
BLU	64.3	101	142	119	326
100 µs					
	Amplitude (nV)				
Animal	P1	N2	P3	P4	N5
COL	125	214	475	334	1,050
TRO	359	543	1,360	686	2,208
OLY	178	220	455	191	705
TYH	53.7	65.9	81.7	73.4	201
SAY	342	640	623	474	1,250
BLU	76.0	106	155	117	377

to stimulus levels > 110 dB SPL. The occurrence of a break-point was not predictable based on the presence/absence of hearing loss nor the frequency of hearing tested. Figure 6 presents example I/O functions for two subjects at 56 kHz—one demonstrating a break-point (TRO, top) and the other without a break-point (TYH, bottom).

Discussion

A statistically significant relationship between click-evoked ABR waveform amplitudes (P1, N2, P3, P4, and N5) and the upper-frequency limit of hearing at the shortest click duration (5 µs) suggests that this electrophysiological method holds potential clinical application for bottlenose dolphin health, particularly in a screening context. Thus, with further study defining normative values for response amplitude based upon upper-frequency limit of hearing, a screening protocol utilizing the click-evoked ABR could be implemented for periodic assessments of hearing range under human care (i.e., comparing it to baseline testing). An additional and significant application could be auditory monitoring for those animals receiving ototoxic antibiotics such as gentamycin or amikacin. However, it should be noted that the click-evoked ABR methodology lacks the ability to determine hearing thresholds at specific frequencies; therefore, the SAM tone-evoked ASSR

Table 3. *Click-evoked ABR:* Waveform latency and interpeak latency values

5 µs								
	Latency (ms)					Interpeak latency		
Animal	P1	N2	P3	P4	N5	P1-P4	N2-N5	P3-P4
COL	1.6	2.4	2.8	3.5	4.0	1.9	1.6	0.7
TRO	1.4	1.8	2.6	3.3	3.7	1.9	1.9	0.7
OLY	1.6	2.0	2.5	3.3	3.7	1.7	1.7	0.8
TYH	1.8	2.2	2.9	3.6	4.1	1.8	1.9	0.7
SAY	1.5	1.8	2.6	3.3	3.7	1.8	1.9	0.7
BLU	1.8	2.0	2.6	3.6	4.0	1.8	2.0	1.0
50 µs								
	Latency (ms)					Interpeak latency		
Animal	P1	N2	P3	P4	N5	P1-P4	N2-N5	P3-P4
COL	1.6	2.4	2.9	3.5	4.0	1.9	1.6	0.7
TRO	1.5	1.8	2.6	3.3	3.7	1.9	1.9	0.7
OLY	1.5	2.1	2.6	3.4	3.7	1.9	1.6	0.8
TYH	1.8	2.2	2.9	3.6	4.1	1.8	1.9	0.7
SAY	1.5	1.9	2.7	3.4	3.8	1.9	1.9	0.7
BLU	1.7	2.1	2.8	3.5	3.9	1.8	1.8	0.8
100 µs								
	Latency (ms)					Interpeak latency		
Animal	P1	N2	P3	P4	N5	P1-P4	N2-N5	P3-P4
COL	1.6	2.4	2.9	3.6	4.0	2.0	1.5	0.7
TRO	1.5	1.9	2.6	3.3	3.7	1.9	1.8	0.7
OLY	1.5	2.1	2.6	3.3	3.7	1.8	1.6	0.8
TYH	1.8	2.4	2.9	3.7	4.1	1.9	1.7	0.8
SAY	1.5	1.9	2.7	3.4	3.8	1.9	1.9	0.7
BLU	1.8	2.1	2.8	3.6	4.0	1.8	1.9	0.8

(or tone-burst ABR) methodology should be used for assessment following any significant findings in the click-evoked ABR response (i.e., outside of test/re-test reliability).

Utilizing the click-evoked ABR methodology as a screening tool could be especially useful in stranded or wild-caught dolphins given its short test time (~1 min as opposed to ~45 min using the SAM tone-evoked ASSR methodology). However, caution must be exercised as extrapolation from findings in bottlenose dolphins should not be assumed to reflect relationships between the ABR and the upper-frequency limit of hearing in other dolphin species. Several considerations must be given. First, the click-evoked ABR amplitude and latencies of other dolphin species to the clicks presented herein should not be assumed to be the same from species to species. Inherent differences in the properties of the auditory system may manifest in latencies and amplitudes that are different than in the bottlenose dolphin. Similarly, there can be large size differences between species, which will also affect click-evoked ABR latencies and amplitudes. Prior to consideration in other species, specific studies relating the click-evoked ABR across individuals within a species should be pursued, and similar relationships between click-evoked ABR characteristics and hearing loss should be explored.

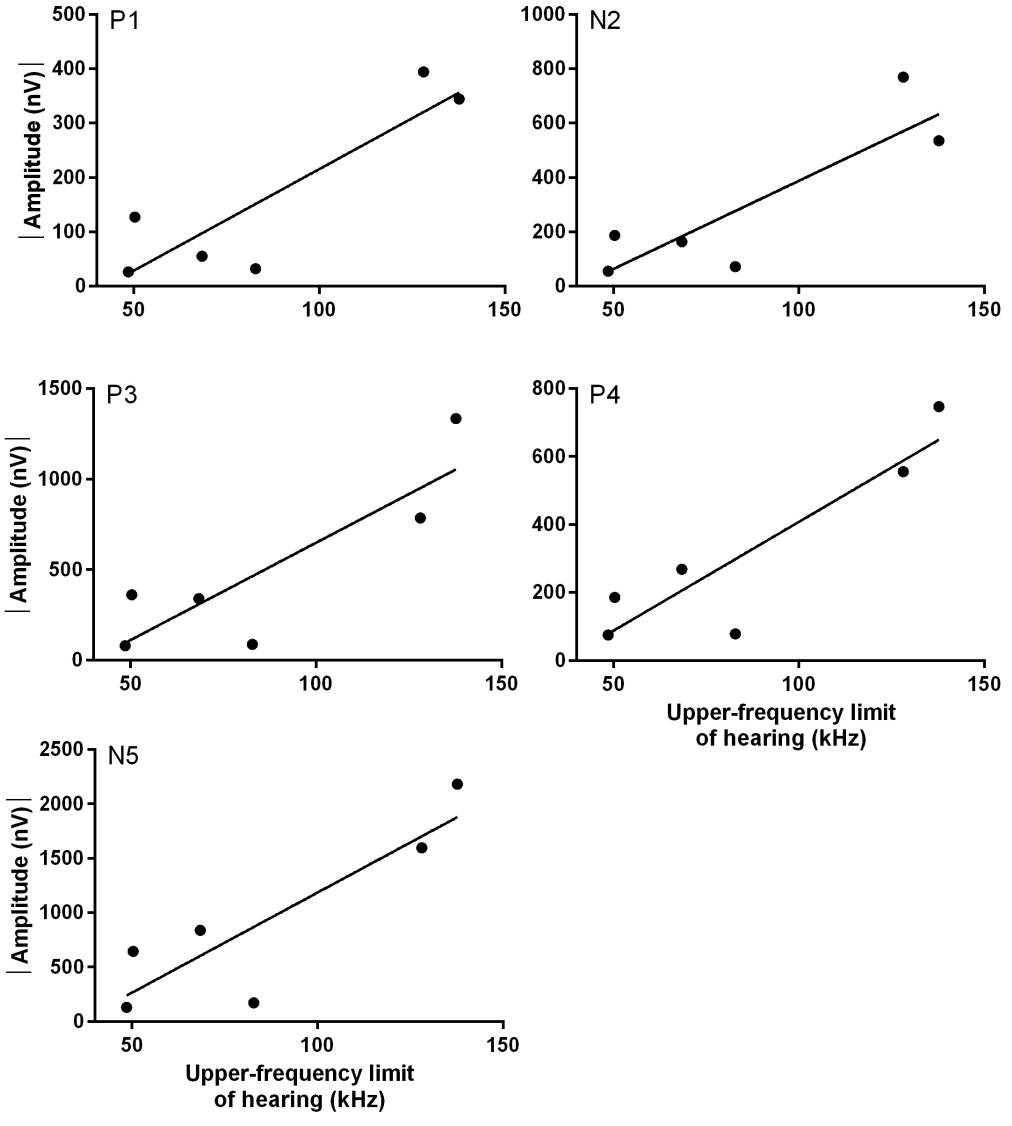

Figure 5. ABR wave amplitude (nV) vs upper-frequency limit of hearing (kHz) for each waveform component (P1, N2, P3, P4, and N5) produced by a 122 dB ppeSPL click of 5 μs duration

Visual inspection of the click-evoked ABR suggests that relationships between the waveforms and hearing capabilities exist. However, with a limited sample size, even though it appears that trends may exist with respect to hearing capabilities and I/O functions, there is enough variability within and among subjects to limit our ability to statistically measure the relationships. It is also feasible that the rapid presentation of click stimuli used in this study resulted in some suppression of the ABR amplitude; nevertheless, the stimulus was the same across animals, and the relationships between waveform amplitude and the upper-frequency limit of hearing should hold. The relationships held when considering several wave amplitudes across different click durations (P1, N2, and P4). It is uncertain as to why the relationship between the upper-frequency limit of hearing and waves P3 and N5 lost significance with the increasing click duration; nevertheless, the same relationship trended with P3 and N5, and the significant relationship possibly would have held with a larger sample size. The click stimuli used in this study were not ideal for this

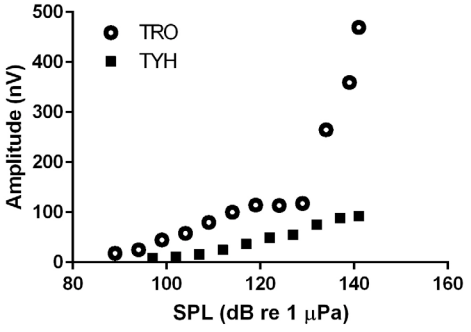

Figure 6. I/O functions derived from 56-kHz sinusoidal amplitude-modulated (SAM) tone-evoked auditory steady-state responses (ASSR) for TRO (top, break-point) and TYH (bottom, no break-point)

type of testing because of the frequency-specific variability in the transmission voltage response of the projector. Furthermore, the longer duration clicks produced a *double-click phenomenon*—that is, two clicks produced by the onset and off-set of the voltage spike. This resulted in spectral rippling that also contributed to an uneven distribution of acoustic energy across the bandwidth of interest. Prior work has demonstrated the contributions of specific frequency bands to the formation of the ABR, which corresponds to contributions from different regions along the cochlear partition (Popov & Supin, 2001). Optimal testing should therefore be sought by attempting to obtain equivalent stimulation of the cochlea across the bandwidth of interest. Stimuli such as clicks with adjusted, equivalent acoustic energy across the bottlenose dolphin's auditory filters, along with a lower presentation rate, could be used to refine the current methodology and provide a better indication of frequency-specific cochlear pathology (Finneran et al., 2015). However, further study is needed to establish normative data by species for widespread use in the marine mammal veterinary clinic, research complex, and/or field (i.e., sample sizes need to be increased to provide more statistical power to exploratory analyses).

Many of the subject factors contributing to the variability of hearing in humans presumably also affect click-evoked ABR variability in the bottlenose dolphin such as age (i.e., neural development), gender, body temperature, ototoxic medication(s), noise exposure, and hearing sensitivity. Similarly, the interaction of these factors may account for much of the variability observed in the frequency-specific I/O functions. High-frequency hearing loss with age, defined as presbycusis, has been documented in the bottlenose dolphin (Houser & Finneran, 2006b) and likely contributes to decreased ABR amplitudes and increased wave latencies (exhibited by subjects OLY, TYH, and BLU in this study). However, similar to terrestrial mammals, audition is also a genetically regulated process in the bottlenose dolphin (i.e., passed from parent to offspring) (Houser & Finneran, 2006b), which could explain some aspects of the variability observed in the ABR waveforms and I/O functions observed herein. For example, genetic processes could explain the lower-than-expected upper limit of hearing in COL, who is much younger (13 y) than expected for an animal that might experience presbycusis. The father of COL also demonstrated abnormal hearing (dolphin No. 30 in Houser & Finneran, 2006b). Genetic etiology is further supported by the fact that COL has had no major illnesses throughout his lifetime and has never been prescribed ototoxic medications known to result in high-frequency hearing loss in at least one odontocete (*Delphinapterus leucas*) for which aggressive treatment was required (Finneran et al., 2005). Noise exposure is also a subject factor known to cause high-frequency (typically) sensory hearing loss, but due to the fact that all subjects included in this study live in the same area, it is assumed their noise exposure history is similar and that there is nothing remarkable about the noise exposure history of COL.

Given promising relationships between click-evoked ABR waveform amplitudes and the upper-frequency limit of hearing at the shortest click duration (5 μs), this methodology holds promise as a clinical tool for a variety of test environments in which expedited assessment of an odontocete's frequency range of hearing is desirable. Although future study delineating normative values for waveform amplitude based upon upper-frequency limit of hearing is necessary, the click-evoked ABR could provide an efficient and informative hearing screening tool.

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