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Hearing pathways and directional sensitivity of the beluga whale, *Delphinapterus leucas*

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ABSTRACT

Odontocetes are believed to receive sounds primarily through the pan bone region of the lower jaw although much variation in jaw morphology exists among species. In order to further examine this jaw hearing hypothesis we tested the head receiving sensitivity and directional hearing of a beluga whale, *Delphinapterus leucas*. Hearing thresholds were measured using auditory evoked potentials (AEPs). The subject proved to have highly directional hearing for far-field click stimuli similar to that of bottlenose dolphins and more directional than the harbor porpoise. For near-field jawphone stimulation, the beluga's lowest thresholds were found when click stimuli were presented at the rostrum tip (76 dB re: 1 µPa) although thresholds from the pan bone region stimulation were only 2–3 dB higher. Stimulation at and behind the external auditory meatus were elevated by nearly 20 dB. Stimuli presented at the surface of the melon did not generate detectable AEP responses, although sound levels of up to 142 dB were employed. Latencies of responses were generally shortest for meatal stimulation and increased with distance. Results support a shaded receiver model for odontocete hearing but how received sounds are filtered and shaded may depend on species. We also suggest that odontocete hearing thresholds are not necessarily lowest through the pan bone region. Rather, hearing pathway variations appear to exist among odontocete species and are at least partially dependent on head morphology.

1. Introduction

Directional auditory sensitivity and sound source localization are crucial aspects of hearing used across taxa to identify the position of predators, prey, and conspecifics. Odontocetes (toothed whales and dolphins) have sensitive underwater hearing and use sound to orient in the marine environment, including localizing sound sources in three dimensional space. Determining the direction and source of a sound can be vital in locating other individuals and localizing targets during echolocation. The directionality of odontocete hearing has been investigated using a variety of experimental methods, including measuring receiving beam patterns (Au and Moore, 1984) and minimum audible angles (Renaud and Popper, 1975), examining variation based on frequency (Supin and Popov, 1993), localizing sensitivities (Møhl et al., 1999) and computational modeling (Branstetter and Mercado, 2006). These studies have revealed a sophisticated hearing system that uses fine scale binaural time difference cues, spectral filtering and amplitude shading to ascertain source positions within an aqueous environment where sound travels rapidly.

Most studies on odontocete hearing and directionality have focused primarily on one species, the bottlenose dolphin, *Tursiops truncatus*. We know much less about the hearing directionality of other species of odontocetes. While auditory structures appear relatively conserved among odontocetes, subtle differences may affect hearing directionality and sound localization. When other odontocete species' auditory capabilities are investigated we often find unique results. For example, the harbor porpoise, *Phocoena phocoena*, has a relatively wide receiving beam, wider than that of the bottlenose dolphin (Kastelein et al., 2005). Kastelein et al., suggested that although this may provide the porpoise a slightly lower signal-to-noise ratio, a broad receiver allows for predator detection and environmental cues from many angles. They also proposed the difference between the porpoise and dolphin hearing directionality was based on (head and body) morphology. More recent investigations in another odontocete, the Cuvier's beaked whale (*Ziphius cavirostris*), have revealed fine scale anatomical differences in their auditory system, speculating adaptations in how sounds are received may be species or even sex related (Cranford et al., 2008a; Cranford et al., 2008b).

Acoustic directionality and localization is a function of several available cues including differences in sound amplitude, time of arrival, phase, and frequency components differing between the two

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receivers, the ears. While hearing anatomy is largely similar between odontocetes, there are apparent slight differences. These differences may affect how sound is filtered, shaded or processed, causing some variation in hearing directionality among odontocete species. To better understand this we must investigate how different species receive sounds.

The beluga whale, *Delphinapterus leucas*, is an ideal subject species to investigate the variation in odontocete hearing directionality for several reasons. First, prior studies that have established baseline auditory information for the beluga including the audiogram, masked hearing thresholds, temporary threshold shift phenomena and auditory filter shapes (Aubrey et al., 1988; Klishin et al., 2000; Finneran et al., 2002).

Second, belugas are unlike other odontocetes in that they do not have fused neck vertebrae (Reynolds and Rommel, 1999) providing the ability to easily turn their head toward a sound source. In terrestrial mammals, turning toward a sound source is an important localization behavior as it allows the use of the pinnae to 'filter' high frequencies and thus use spectral cues to determine sound directionality (Butler, 1975; Butler, 1986). Although cetaceans have lost their external pinnae, they likely use the morphology of the head to shadow and filter frequencies and help localize sounds (Ketten, 1997; Ketten, 2000). Previous research has shown that bottlenose dolphins and belugas have directional sensitivity (Au and Moore, 1984; Klishin et al., 2000). However, these studies did not measure sensitivities beyond 105° from the animals' azimuth midline axes, despite the fact that odontocetes likely use hearing in all directions. The only study to conduct such methodology used a harbor porpoise as a subject, finding it unexpectedly broadly directional for localization purposes (Kastelein et al., 2005). Based on their ability to turn their head (Reynolds and Rommel, 1999) and preliminary directionality studies (Klishin et al., 2000), it appears that a more detailed study of beluga hearing might reveal relatively narrow directional hearing.

Finally, belugas do not have a protruding rostrum and lower jaw, as found in dolphins. The best supported hypothesis of an odontocete sound receiver is the use of the lower jaw (Kobler et al., 1992). Sound is thought to enter the head through fat bodies of the lower jaw which have an impedance close to that of sea water (Varansi and Malins, 1972; Koopman et al., 2006). The dolphin lower jaw ends in a thin bony plate termed the pan bone which sound passes through at the proximal end where the bone is relatively thin. Internal mandibular fat bodies then likely conduct the sound to the bony ear complex although it is not yet established how sound is actually transmitted into the auditory bulla (Norris and Harvey, 1974; Ketten, 2000). This lower jaw hearing hypothesis has been supported by several studies demonstrating that thresholds are lowest when a localized sound source is placed near the pan bone region of the bottlenose dolphin lower jaw (McCormick et al., 1970; Bullock and Budelmann, 1991; Møhl et al., 1999). While it is likely that odontocetes generally receive sound in this manner, there are obvious differences in head morphology across species. This may tailor niche-related subtle differences in how sound is received, for example the point of maximal jaw sensitivity. Examining differences in sound reception in odontocetes other than the bottlenose dolphin remains unexplored.

In this study the auditory evoked potential (AEP) method was utilized to address questions of beluga hearing sensitivity and directionality. The AEP technique provides a means to investigate the hearing of odontocetes both rapidly and passively (Nachtigall et al., 2005; Mooney et al., 2006; Nachtigall et al., 2007). Measurements can be made with minimal or no animal training and therefore allow more questions to be addressed. A preliminary audiogram was established to determine the subject's baseline hearing. Thresholds were then measured up to 180° relative to the animal's anterior-posterior azimuth midline axis to evaluate directionality of hearing. Finally, regions of best sensitivity were examined across the head of the whale.

2. Materials and methods

2.1. Subject and timeline

The subject of this study was Yulka, a nine-year-old adult female beluga whale (*Delphinapterus leucas*) housed at l'Oceanogràfic marine park, Valencia, Spain. The animal had been at the facility for three years, was 3.73 m in length and weighed approximately 600 kg. Yulka's facilities included four separate connecting pools, two of which were public display areas, and a total water volume of 3582 m^3 with 800 m^2 of water surface. The pools were filled with cooled, filtered saltwater pumped in from the nearby Mediterranean Sea. Data sessions were conducted for 6 continuous days from April 29-May 4 2007 in three experimental situations: a) a baseline audiogram, b) thresholds of broadband clicks at three azimuth angles, 0°, 90°, and 180° and c) click thresholds at 5 jawphone source positions on the animal's head. The first two experiments generally overlapped in procedure and thus the methods are explained together with any differences highlighted. The jawphone source position experiment, which examined relative sensitivity across the whales head, differed slightly in methods so it is explained separately. Within the results and discussion, the three experiments are presented in separate sections. These experiments required the cooperation of a well-trained subject and consequently this investigation was limited to one experimental animal. In order to ensure that our data was not strongly influenced by an individual difference, we compared the baseline audiogram collected to that of other beluga whales and odontocete cetaceans to demonstrate the subject heard normally.

2.2. Experimental set-up

Measurements were made in the rear of the large exhibition pool which was the largest beluga pool in the park that had a volume of 2,699 m^3 and was 5 m in depth (Fig. 1a). Although asymmetrical in shape, the tank was approximately 25 m in diameter. Two columns in the pool supported the dome shaped roof of the facilities. All wall and column surfaces were irregular and were created to imitate the look of an ice environment but were made of concrete covered by white and blue epoxy. The size of the facilities and irregular wall shape made for a relatively free-field environment with limited interfering acoustic reflections from the sides of the tank. The tank water returned to the filtration system by four skimmers on the sides of the tank. These skimmers produced a constant low frequency noise that had peak values (107 dB re: 1 uPa RMS) in the range of 450 – 650 Hz but dropped to the measurable noise floor by 10 kHz. To record the pool's total background noise, ten 1-s noise files were recorded using a custom LabView program and National Instruments PCMCIA-6062E DAQ card (Austin, TX, USA) implemented into a laptop computer. The ambient sound was collected using a Reson 4040 hydrophone (Slangerup, Denmark) connected to a Krohn-Hite filter (Brockton, MA, USA) which was connected to an NI SCI-68 break-out box and the DAQ card. Noise files were sampled at 450 kHz. The filter amplified the incoming records by 20 dB and provided a low pass filter at 200 kHz to prevent aliasing, although the resonance cut-off of the hydrophone was approximately 100 kHz. Background levels were then referenced using an 8 kHz tone calibrated at 119 dB re: 1 μPa RMS. The ten noise files were compared to ensure no extraneous signals were present, however only one file was plotted (Fig. 1b). Ambient noise proved to be low, below the sensitivity of the acoustic recording equipment (68 dB re: 1 $\mu Pa^2 Hz^{-1}$) at frequencies greater than 10 kHz. Below 10 kHz a low level of background noise was apparent although generally not of concern because frequencies of interest were 8 kHz and higher. Low-noise situations such as these are valuable situations for conducting absolute hearing threshold measurements (Au et al., 2002).

During the experiment the animal was stationed at the water's surface, 1.5 m parallel to the pool wall located to her left. The nearest

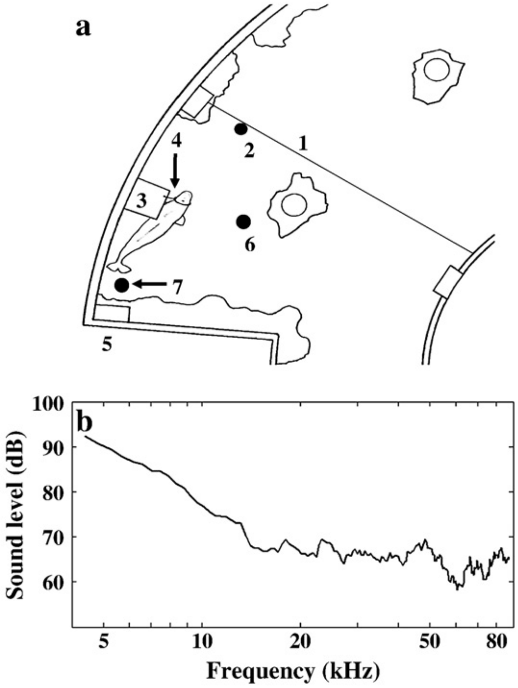

Fig. 1. a. Experimental set-up at the rear of a large exhibition pool. 1, rope strung above the pool; 2, transducer at 0° azimuth plane, hung from the rope and directly in front of beluga; 3, trainers station; 4, beluga whale in hoop; 5, nearest skimmer; 6 and 7, directionality experiment's transducer positions at 90° and 180°, respectively, in the azimuth plane relative to the subject's anterior-posterior midline axis. b. Tank background noise plotted recorded using a Reson 4040 hydrophone and custom built LabView program that implements a 6062E NI DAQ card in a laptop computer. Noise was sampled at 450 kHz and analysis was made with a 1024 point FFT using a 10-point moving average and plotted in dB re: 1 $\mu Pa^2 Hz^{-1}$.

walls on other sides were 4.5 m behind the animal 30 m in front and 15 m to the right, with a pillar half the distance to the wall. The animal was trained to hold a constant position by stationing in a hoop and touching its melon to a foam target placed in front of the hoop. Because belugas have an extremely flexible neck, the target and hoop were used to ensure that the subject kept her head and body still and facing the transducer. The trainer sat on a wooden platform above and to the left of the whale to closely observe the animal and assure that she maintained a consistent position. During audiogram measurements the projecting transducer was hung from a line that was placed across the pool so that the transducer was 2.15 m in front of the beluga and at a depth of 30 cm. For the directionality experiment, the transducer was suspended at the same location at 0° (i.e., directly in front of the animal along its anterior-posterior axis), but for 90° and 180° the transducer was suspended from a pole and at distances of 2.15 to animal's right and 3.8 m behind in the azimuth plane (Fig. 1a). All distances were measured from the approximate location of the animal's ears while at station and the distance behind the animal was greater to place the transducer behind the animal.

2.3. Acoustic signals and calibration

All signals were calibrated prior to the experiment. The projecting transducer was hung 2.15 m in front of the animal's hoop position, or in the case of the directionality experiment, signals were projected from the 0°, 90° and 180° respective azimuth positions (Fig. 1a). A receiving hydrophone was positioned at 30 cm depth in front of the hoop at the estimated position of the animal's ears. Two transducers were required to project the underwater stimuli: an ITC-1032 (Santa Barbara, CA, USA) was used to project lower frequency tones from 8–32 kHz and a Reson 2130 for higher frequency tones (from 50–128 kHz) and clicks. The receiving hydrophone was a Reson 4040 positioned 1.5 m from the tank wall. The projecting transducers were either 1.5 m (when directly in front of or behind the animal) or 3.65 m from the tank wall (when 90° to the animal's right). The acoustic signals were sinusoidally amplitude modulated (SAM) tones and a 100 μs click, centered at 80 kHz (−3 dB from 91–68 kHz). The calibrated signals were the same stimuli as those presented to the whale during the hearing tests. The synthesized click was specifically designed to optimize a region of the subject's best sensitivity and reflect the prominent energy found in the animal's echolocation click (Castellote and Fossa, 2006). Received sound levels were calibrated at 11 frequencies which were later used to test the animal's basic hearing: 8, 11.2, 15, 23, 32, 50, 70, 80, 90, 100, and 128 kHz. Each of the SAM tone sine waves was transmitted in the tank and the received peak-to-peak voltages ($V_{p\text{-}p}$) were measured with the calibrated hydrophone. This $V_{p\text{-}p}$ was converted to peak-equivalent root-mean-square voltage (peRMS) by subtracting 15 dB. The peRMS was taken as the RMS voltage and used to calculate the sound pressure level (SPL) for that frequency (dB re: 1 μPa). Sound pressure levels of the clicks were measured using $V_{p\text{-}p}$ as is standard to measure odontocete click intensities due to the inherent brevity of the signals (Au, 1993). The sound levels of the clicks and SAM tones were related by integrating the $V_{p\text{-}p}$ over the duration of the respective signals to provide the energy flux density of the signals (dB re: 1 $\mu Pa^2 s$) (Au et al., 2002). Signals projected from the 90° and 180° azimuth positions for directionality experiment were calibrated in the same manner but from their respective transducer positions.

The waveform of the received signals was viewed with a Tektronix TPS 2014 oscilloscope (Beaverton, OR, USA), to confirm that there were no competing reflections produced from other signals or reflections in the tank. In this environment there were no constructing or destructing interferences observed with the transmitted signal. Had these sorts of interferences been present they would have been apparent in SAM reflections on the oscilloscope screen; they would also have been extremely unlikely because of the transient properties of the short SAM tone-bursts.

For the hearing tests, acoustic stimuli were digitally created using a custom LabView program and DAQ card installed in a laptop computer. Both stimulus types, clicks and SAM tones, were repeated or modulated at a rate of 1000 Hz, the previously determined effective rate for beluga whales (Klishin et al., 2000). Stimuli were presented in 19-ms stimulus trains and alternating with 30 ms of silence, and thus presented at a rate of 20 s^{-1}. Signal trains were played 1000 times, thus each trial lasted approximately 50 s. Lower frequency stimuli (8–32 kHz) were synthesized using an update rate of 256 kHz while those of 50 kHz and above, as well at the clicks, had an update rate of 512 kHz. The signals were sent from the computer to an HP-Attenuator 350D that could attenuate in 1-dB steps. The oscilloscope was used to monitor the outgoing stimuli from the attenuator to the projecting transducer.

2.4. AEP Measurements

Auditory evoked potentials were collected using passive, gold EEG electrodes imbedded in custom-built latex suction cups. The electrodes were standard 10-mm EEG electrodes, the same type used for human EEG collection. The suction cups were placed on the animal at the beginning of each session with standard conductive gel. The active electrode was attached about 3–4 cm behind the blowhole, slightly off to the right and over the brain. The reference electrode was attached posterior to the active, on the animal's back, and near the third ground electrode. Placement of the active electrode proved quite challenging as the mobile head and skin surface of the beluga allowed the animal to easily dislodge the suction cup. Thus the first research session was dedicated to determining the best region for cup placement in regard to a position where it would not be displaced and yet still received the

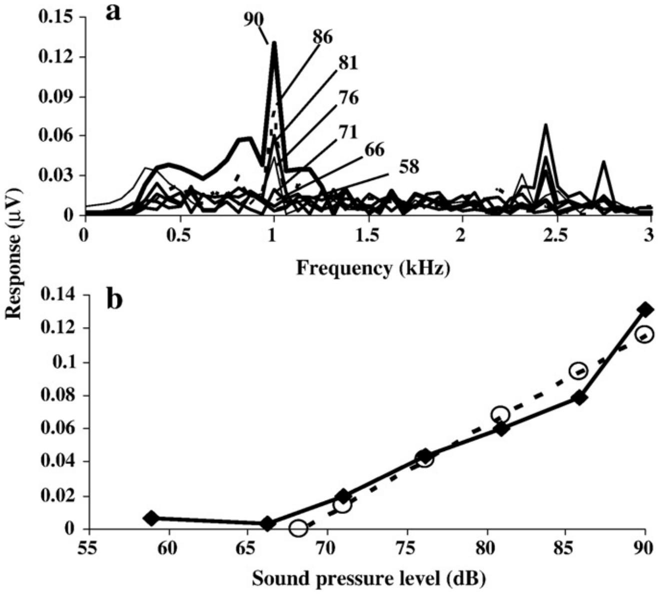

Fig. 2. a. Fourier transform of the envelope following responses measured using 11.2 kHz SAM tones as the carrier frequency, a 1000 Hz modulation rate and stimuli intensities from 90 to 58 dB re: 1 μPa. Sound pressure levels of stimuli are labeled in dB indicating their corresponding AEP response spectra. b. Plot of the peak value of each Fourier spectra at the 1000 Hz modulation frequency (solid line-diamonds) for each SPL presented and best fit regression (dotted line-open circles) used to determine the threshold at 11.2 kHz.

best AEP signal. The optimal position proved to be several cm behind the blowhole but just anterior to creases from the beluga's neck. For the audiogram and subsequent directionality experiment the animal rested at the surface with its blowhole and the electrodes remaining out of the water, while most of its head was underwater. This configuration maximized data collection efficiency and AEP signal strength.

The electrodes were connected to a Grass CP511 bio-amplifier and filter (West Warwick, RI, USA), set to amplify the AEPs by 10,000x and filter the responses between 300 and 3000 Hz. The responses were then run through a Krohn-Hite 3384 filter with the same filter settings to further protect against aliasing. The amplified and filtered responses were transferred to an analog input of the same DAQ card in the same computer and digitized at 16 kHz. In order to extract the AEPs from noise, 1000 response records were collected and averaged for each trial (one frequency and SPL). Each AEP record was 26 ms in duration and began simultaneously with stimuli presentation.

2.5. Threshold measurements and AEP analysis

The procedure was identical for all threshold measurements and each threshold was measured once. Before each session, a carrier frequency or click was selected as the stimulus and the initial SPL of the stimulus was determined for the first trial. For the following trials stimulus intensity was determined on the AEP responses in the prior trials but generally SPLs were decreased in 5–10 dB steps between trials until no response was visible for 2–3 trials. Each threshold took approximately 5–10 min and 2–4 thresholds were collected each session. Usually 2 sessions were collected each day. Frequencies and start intensities for the audiogram were determined by referencing a previous beluga AEP audiogram paper (Klishin et al., 2000). Click start intensities were determined by the subject's measured audiogram thresholds at the click center frequency (80 kHz). Stimulus intensity levels began 20–30 dB above the estimated threshold values. An average of 7 intensity levels were presented for each of the 19 different thresholds measured.

In odontocetes, a clear and defined SAM tone or click train produces an AEP response that ‘follows' the envelope of that stimulus. This response has been termed the envelope following response (EFR) (Supin et al., 2001). In this experiment a 16-ms portion of the EFR was fast Fourier transformed (FFT) for each frequency and intensity level (Fig. 2a). This window contained a whole number of response cycles. The 256-point FFT provided a response frequency spectrum of the data where a peak reflected the energy received, or the animal's physiological response to the 1000 Hz modulation rate. A larger EFR response was reflected as a higher FFT peak value. The peak FFT amplitude at the modulation or repetition rate was used to estimate the magnitude of the response evoked by the SAM stimulus.

For each of the frequencies or projecting transducer placements, the FFT peak at each stimulus intensity level was plotted as response intensity as a function of the SPL of the stimulus (Fig. 2b). A linear regression addressing the data points obtained was hypothetically extended to zero, the theoretical point where there would be no response to the stimulus. This zero point had to be extrapolated because of the low level of biological electrical noise always present in the records that would mask the actual zero point. However, by estimating the zero response level it was possible to predict the threshold for each frequency and transducer placement presented to the animal. Analysis was conducted using Excel, Matlab, and Minitab software.

2.6. Jawphone presented stimuli

To measure head relative sensitivity to click stimuli we used a custom built jawphone transducer. The piezo-ceramic transducer element was imbedded in a latex suction cup that was easily and gently attached to the beluga's skin. The transducer's frequency response was from 40–100 kHz. The jawphone was calibrated in the free- and far-field at standard 1 m distance from a receiving hydrophone. Click SPLs were determined by $V_{p\text{-}p}$ in the manner previously described. In this way thresholds from jawphone measurements could be compared to far-field thresholds while recognizing the differences between free-field and contact measurements (Cook et al., 2006; Finneran and Houser, 2006).

The subject's head and the jawphone were kept out of the water to ensure that the surface of the jawphone was the only sound pathway to the animal. Therefore, the animal was stationed in varying positions, from that in the previous experiments (for melon placement), to lying on its side for pan bone, meatus and behind meatus placements, and then to holding vertical in the water column with its head out of the water for the lower rostral jawphone placement. Electrode placements could generally be kept constant except when

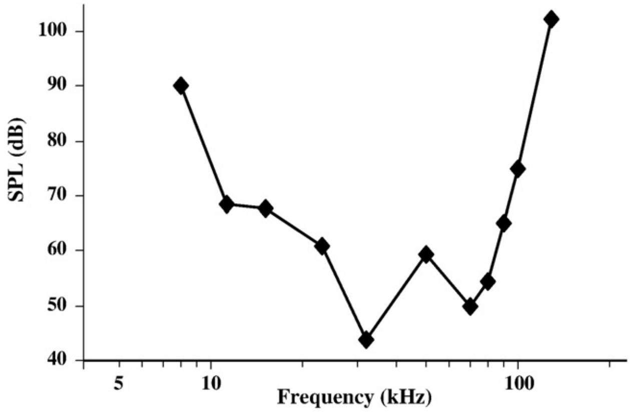

Fig. 3. AEP audiogram of the beluga whale subject stationed at the surface. Thresholds in dB (re: 1 μPa) were measured from 8 to 128 kHz using SAM tones.

the animal was vertical. When the animal was vertical, the reference and ground electrodes were moved anteriorly to keep all electrodes out of the water. Moving the ground and reference electrodes to comparable locations had no effect on threshold determination, based on initial measurements establishing the best electrode placements. Similar results have been established in prior experiments which demonstrate that if the recording electrode is kept constant, moving the reference and ground have minimal effects on AEP magnitude or latency (Beattie et al., 1986; Finneran and Houser, 2006; Houser and Finneran, 2006).

In order to determine the regions of 'best' response, two primary variables were analyzed. The first was the relative threshold of response at the varying positions. The second variable was latency of the peak response. These were measured by establishing the time (ms) between stimulus onset and the point of maximal change in neuronal firing. This was determined by measuring the time to the rising front of the most prominent peak (IV). Generally this was measured for the first 2–3 AEP responses, i.e., the responses to the maximal SPL presented and 1–2 attenuation levels below because peak IV was unambiguous at these levels.

3. Results

3.1. Audiogram

The baseline audiogram in the free field revealed that the subject had quite sensitive hearing with thresholds below 60 dB re: 1 μPa between 32 and 80 kHz and below 70 dB at 11.2 and 90 kHz (Fig. 3; Table 1). As is typical of odontocete hearing thresholds they increased gradually at lower frequencies (<32 kHz) and more steeply for higher frequencies to the least sensitive threshold measured of 102.8 dB at 128 kHz. The whale also had a distinct notch in the audiogram at 50 kHz.

3.2. Directional sensitivity

Thresholds were also measured using far-field broadband clicks with the source placed in several positions. Directly along the anterior azimuth midline, with the source directly in front of the animal, click thresholds were measured at 85 dB (Fig. 4). The threshold at 90° relative to the midline and the animal's ears was 105 dB, dropping off 20 dB from along the animal's anterior/posterior axis. At 180°, or directly behind the animal, the threshold was an additional 9 dB higher or 114 dB.

3.3. Received sensitivities from jawphone stimuli

The beluga's sensitivity and AEP latency were measured at various locations on the animal's head (Fig. 5a) using a contact jawphone for stimulus presentation. The same broadband clicks as used in the directionality experiment were also presented — but they were presented via the jawphone. The region of maximum sensitivity (76 dB) was found to be at the tip of the lower jaw (2) of the animal (Fig. 5b). The pan bone area (3) was found to have a slightly higher but similar threshold (78 dB). Sensitivities dropped off considerably at the

Table 1
Auditory evoked potential (AEP) thresholds of a beluga whale in dB (re: 1 μPa)

Frequency (kHz)	Threshold (dB)
8	90.2
11.2	68.5
15	67.9
23	60.8
32	43.9
50	59.4
70	49.8
80	54.3
90	65.2
100	75.0
128	102.4

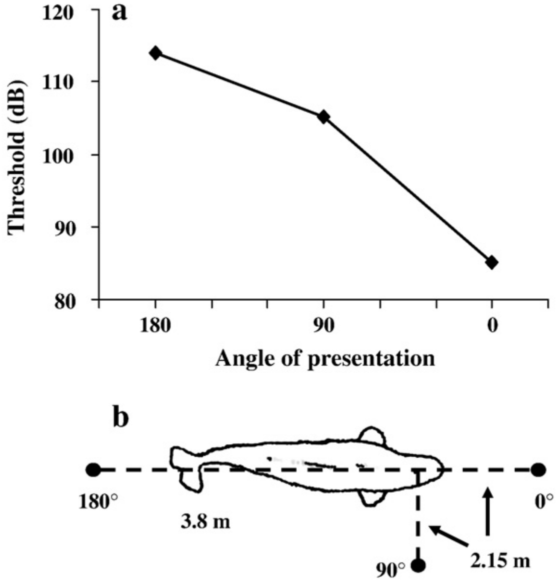

Fig. 4. a. Response thresholds to click stimuli presented at azimuth angles of 0°, 90° and 180° where 0° and 180° are along the anterior-posterior midline and 90° is at a right angle to the beluga's external auditory meatus. Thresholds are presented in dB (re: 1 μPa) and click levels were measured in V_{p-p} and stimuli were presented in the far-field. b. Sketch of the directivity experiment set-up. Indicated are the distances (both for the calibration and the experiment) from the transducer to the whale's ears.

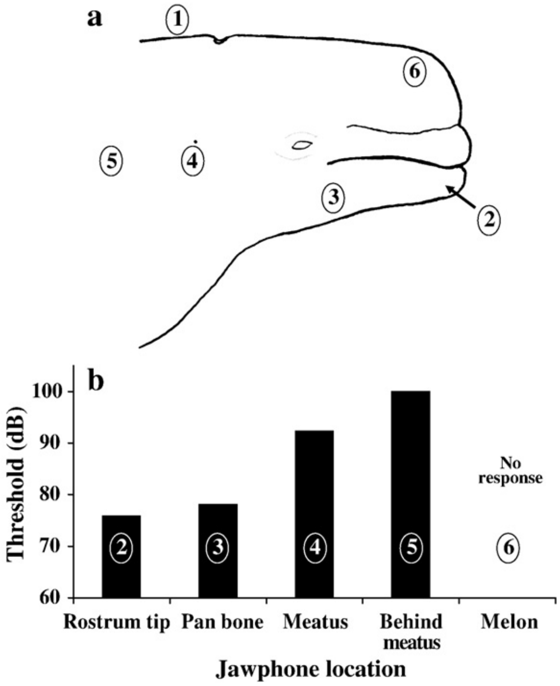

Fig. 5. a. Diagram of beluga's head for AEP recording with points of stimulation indicated. 1, location of active AEP electrode; 2, rostrum tip; 3, pan bone; 4, external auditory meatus; 5, behind meatus; 6, melon. b. Response thresholds to click stimuli based on 5 different jawphone placements. Thresholds are presented in dB re: 1 μPa using p-p SPLs measured at 1 m.

position of the external auditory meatus (4) and 12 cm behind the meatus (5), with thresholds of 92 and 100 dB respectively. Interestingly, no response at all could be detected when the jawphone was placed on the whale's melon (6), despite SPLs of 142 dB presented to the animal. This lack of response from the melon presentation was further demonstrated when the subject briefly lowered her melon and thus the jawphone into the water during the trial at 130 dB (Fig. 6). A response was immediately detected by a peak developing in the FFT at the modulation frequency of 1 kHz. The trial was then quickly stopped. When the 130 dB trial was repeated, ensuring that the jawphone remained out of the water, no response was detected and the FFT reflected a minimum at the modulation frequency.

In order to determine the latency of the AEP response the precise waveform characteristics had to be identified. The 19 individual near-sinusoidal EFR waves produced from higher intensity stimuli were examined. By counting backwards from the last wave (found at approximately 25 ms after stimulus and recording onset) it was possible to determine the four initial AEP waves that were a response to the onset stimulus (Fig. 7a). These four initial waves and the subsequent EFR were similar to those of a previously measured beluga whale AEPs (Klishin et al., 2000). After the initial waves were identified, their latencies were measured from stimulus onset. In this manner, AEP latencies were determined for the four locations in which the jawphone generated AEP responses. The latency of the only the three largest waves (II, III and IV) were measured and only at relatively higher stimulus intensities in order to avoid ambiguous measurements of responses that were close to background noise levels. Because they showed the largest response (up to 1 μV), the patterns of wave IV were considered the most faithful measure of AEP latency, although all waves measured showed the same general trend. Minimum latencies for all three waves were found when the jawphone was placed at the external auditory meatus or 12 cm behind the meatus (Fig. 7b). Latencies increased at pan bone and rostrum tip placements respectively. For wave IV, response latency was shortest from the meatus at 6.5 ms. Waves II and III had minimum latencies of 4.1875 and 5.375 ms respectively, measured from 12 cm behind the meatus. Maximum AEP response latencies were all measured during rostrum tip stimulation and measured 4.875, 5.1825 and 6.75 ms for waves II, III and IV respectively. The difference between maximum and minimum latency was quantified for each wave. The greatest difference in latency duration was 0.6875 ms, for Wave II, measured as the difference between rostrum tip stimulation and 12 cm behind the meatus. Wave IV had considerably less variation in latency differences (0.25 ms) between stimulation points, found between the meatus and rostrum tip.

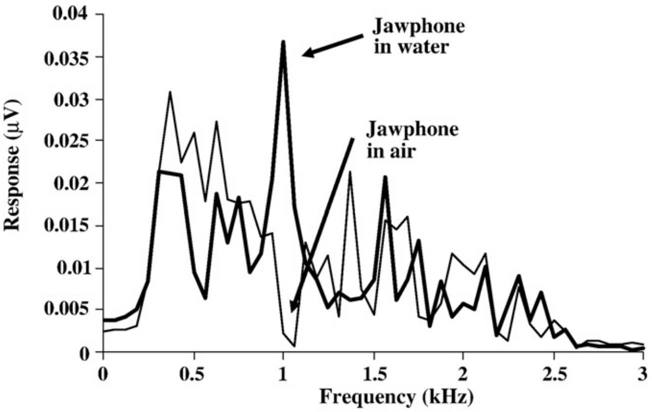

Fig. 6. Fourier transform of beluga EFR when click stimuli were presented from the melon. Bold line with peak at 1 kHz reflects when jawphone was dipped into the water and indicates subject's auditory system heard and was following the clicks. The finer line was when the jawphone remained out of the water for the entire record and indicates the animal did not detect the click stimuli. Both situations are indicated on the graph. Stimuli for both records were presented at 130 dB.

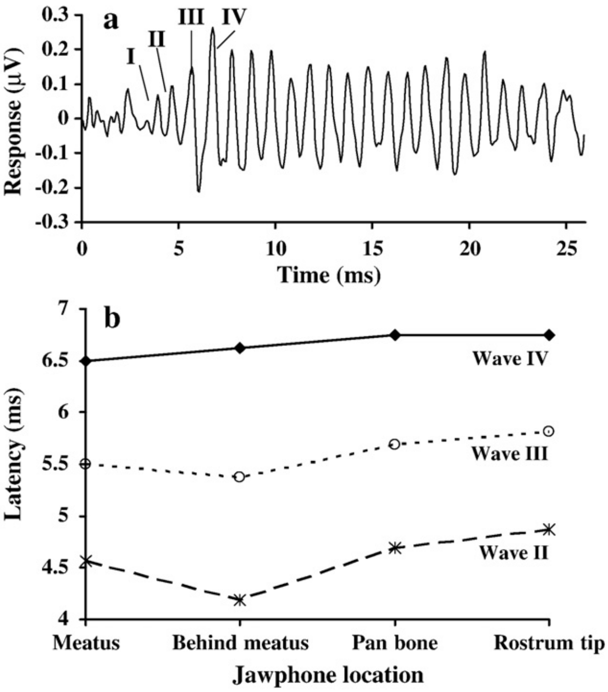

Fig. 7. a. Four initial AEP (I–IV) waves and succeeding EFR to click stimuli presented at the pan bone region using a SPL of 105 dB re: 1 μPa. Waves II, III and IV were used. b. Latency of response (ms) to various jawphone locations using the three response waves of greatest amplitude, II (bottom-dashed line), III (middle-dotted line) and IV (top-solid line).

4. Discussion

4.1. Audiogram

The free-field AEP audiogram revealed beluga whale thresholds of greater sensitivity than previously published. Although hearing thresholds of the beluga have been reported before, previous studies found differing results. Either beluga thresholds were shown to be less sensitive (n=1; Klishin et al., 2000), the studies limited focus to lower frequencies (n=3; Aubrey et al., 1988), or the research was not published in peer-reviewed journals (n=2; White et al., 1978). However, these differences in thresholds were minor and likely reflect a combination of individual (i.e. Klishin et al.,) and methodological differences (e.g. a focus on lower frequencies). Further, of the two published audiograms that encompass low and high frequencies (Klishin et al., 2000; this study), both are AEP audiograms. This seems to highlight the relative emphasis on AEPs for current marine mammal hearing work. If the audiogram here is compared to the audiogram by White et al., the thresholds actually track each other very closely. Both studies found overall low thresholds, near 45 dB for some frequencies and a steep high-frequency cutoff near 100–128 kHz. The two studies also revealed two highly sensitive regions (<60 dB), a lower frequency region centering near 32 kHz and a higher frequency region from 70–80 kHz. Between these frequency bands, both studies found a clear notch at approximately 50 kHz. Unfortunately, while the data of White et al. is the first report of beluga thresholds and reflects the audiogram presented here, their data was only published as a technical report thus caution is required when considering the results. Additionally, whether this notch is found in all belugas cannot be certain, but to be observed in three of the four animals for which there are complete audiograms was intriguing.

4.2. Directional sensitivity

Klishin et al. (2000) measured the thresholds of a beluga as a function of sound source azimuth using tone pips and clicks from 0–105° difference from the animal's anterior-posterior azimuth midline. Thresholds were found to be elevated by approximately 20 dB at 90° regardless of stimulus. Their results were quite similar to our study which used broadband clicks and found a 20 dB increase in thresholds from 0° to 90°. Continuing to an azimuth of 180°, or along the anterior-posterior midline but behind the animal, the threshold dropped an additional 9 dB. This near 30 dB drop in sensitivity from anterior to posterior along the animal's midline is quite dramatic and reflects a highly directional hearing system.

This is in contrast to the harbor porpoise, the only other odontocete in which 180° sensitivity has been measured (Kastelein et al., 2005). These porpoise thresholds were measured using frequency modulated tones (16, 64, and 100 kHz) in a 360° horizontal plane around the porpoise. The animal appeared to have greater relative sensitivity at higher angles (less directional) with sensitivities dropping off by no more that 15 dB from 0° to 180° regardless of the frequency tested. This suggests that the mechanisms for producing directional hearing may not work as well for smaller odontocetes (e.g. porpoises or perhaps calves) with shorter distances between receivers (i.e. the ears or jaw fats). A larger distance between receivers may provide a more directive beam, especially at higher frequencies where phase, amplitude and spectral differences, potential cues for sound source localization, are likely greater. The beluga's body size may also have shadowed the clicks somewhat indicating body and orientation play important roles in directionality. Similar shadowing by the head and body has been demonstrated in terrestrial mammals and plays a greater role at higher frequencies which are more easily shaded (Brown, 1994). The same is likely true for marine mammals and supports why odontocetes have greater directional hearing at higher frequencies (Au and Moore, 1984; Klishin et al., 2000; Kastelein et al., 2005). Thus a better examination of beluga hearing directionality across frequencies and angles would test this hypothesis.

In order to map the bottlenose dolphin receiving beam pattern in front of the animal, Au and Moore (1984) used 2-s tones of 30, 60, or 120 kHz up to 90° in the horizontal plane, although not all frequencies were tested at all angles. At 90° and 30 kHz, thresholds dropped only 10 dB. Higher frequencies appeared to be narrower in receiving beam thresholds however, they were not measured beyond 50° thus it is difficult to draw conclusions. Higher relative thresholds at 90° for the beluga may indicate that it is more directional in its hearing than the dolphin. Unfortunately, with limited data these comparisons are purely speculative.

Based on the results of this study, the beluga seems to have more directional hearing than the harbor porpoise and potentially similar directionality for the bottlenose dolphin. It is possible that the unfused vertebrae, and thus the highly movable head, of the beluga have allowed for adaptations of highly directional hearing. The ability to move and rotate the head has resulted in good directional hearing in terrestrial mammals because sound localization is enhanced by turning toward the source (Brown, 1994). The size and/or shape of the beluga body and head are considerably larger than that of the harbor porpoise. This may serve to shadow off-axis high frequency signals and result in greater directionality. Further, head morphology including acoustic fat location, material composition likely play an important role in sound wave guiding, like that of the terrestrial mammal pinna (Brown, 1994; Müller, 2004). Narrow receiving beams, i.e. a directional receiver, will also enhance signal-to-noise ratios (S/N). During echolocation, when echoes are returning primarily from directly ahead of the animal, lower noise would potentially allow easier echo detection, especially in high clutter environments. Finally, hearing directionality may aid the belugas in detecting and localizing the acoustic signals of conspecifics. Belugas are highly social animals with a complex repertoire of social sounds which range in temporal and frequency components (Castellote and Fossa, 2006). Acute directional hearing for higher frequency and broadband signals (Branstetter and Mercado, 2006) would likely aid in using acoustics to maintain fine scale cohesion and coordination by enhanced localization capabilities. Broad directional sensitivities at lower frequencies would allow for detection of conspecifics at longer ranges when signals may be attenuated. Again, frequency and hearing directionality should be investigated further.

4.3. Receiving pathways from jawphone stimuli

The lowest thresholds were measured when the jawphone was placed at the tip of the lower jaw (rostrum tip) and pan bone region. This is both in contrast and agreement to what was previously found with the bottlenose dolphin (Møhl et al., 1999). Møhl et al., used a jawphone to project clicks to a bottlenose dolphin. The jawphone was moved around the animal's head and lower jaw, measuring AEP responses to various transducer placements. The authors found that the dolphin's rostrum tip was not very sensitive while the pan bone region was highly sensitive. The magnitude of lower rostrum tip sensitivity measured here was unexpected and may indicate there are acoustic fat channels which begin at the beluga rostrum tip that effectively guide sound to the ears. A similar pathway has been recently propsed in the Couvier's beaked whale (Cranford et al., 2008a). In the dolphin, these channels do not start as far anterior on the rostrum but good sensitivity is found in several locations along the outer part of the jaw (McCormick et al., 1970; Bullock and Budelmann, 1991; Møhl et al., 1999). Out data also reflects good reception of sound in the pan bone region, supporting Norris's jaw hearing hypothesis in the beluga (Kobler et al., 1992). While we cannot fully eliminate the possibility of bone conduction from the rostrum tip, we find this idea unlikely as it has not been supported in other studies (Møhl et al., 1999; Ketten, 2000) and simple impedance matching from water to acoustic fats transfers sound waves better (Varansi and Malins, 1972) and would excite a greater AEP response than from water-to-bone. The stimulation point we chose on the rostrum tip was also centered on the lower jaw. If sound is effectively conducted from this region, it may stimulate both ears (as opposed to primarily ipsilateral stimulation from the pan bone region) (McCormick et al., 1970) and excite a greater relative AEP response. The relative sensitivity from this region may also play a role in directional hearing and sound localization. For example, sound from a source directly in front would be primarily received on the rostrum tip (and perhaps both pan bone areas) and then conducted to both ears well. But greater shadowing, and thus amplitude differences, may occur if sound is primarily received from the side and other locations. It should also be noted that thresholds were only measured with broadband clicks. By using tones, thresholds and relative sensitivities to jawphone placement may change (Popov et al., 2008). If so, the spectral properties of a perceived sound would depend on the direction of the sound source and demonstrate how spectral properties may influence sound reception and localization.

Our lack of detected responses from melon stimulation was unexpected and indicates that the beluga melon is not a good acoustic receiver, at least for this individual and this set of circumstances. It appears that the beluga bulla and ears are likely well insulated from the melon, perhaps to reduce the hearing of, and masking by, self-generated echolocation clicks as has been shown by Supin et al. (2006). As evoked potential responses recorded after from melon stimulation were measured in the bottlenose dolphin (Bullock et al., 1968; Møhl et al., 1999), this may indicate that there is some subtle variation in the melon morphology of odontocetes and the bottlenose dolphin may not be as effectively insulated from its own clicks. Or slight differences in transducer placement on the melon might greatly affect how sound is propagated through this tissue. Thus differences in responses between the beluga and dolphin are not species variations but experiment methodological differences and reinforcing the beamforming effect of the odontocete melon (Au et al., 2006).

The latencies of AEP responses were found to generally increase with distance from the external auditory meatus. Precise beluga head morphology has not been sufficiently described, but if tympanic bulla and middle ear locations are in locations similar to delphinids, they are roughly internal from the meatus. However, latencies of waves II and III and the mean latency of all waves were found to be fastest from 12 cm behind the meatus. This may also simply be data scatter as wave IV, the most prominent wave, reflects a clear trend of increasing latency from meatus to rostrum tip. It may also indicate that the tympanic bulla is slightly posterior from the meatus and oriented more toward the posterior, or that a sound pathway from behind the meatus enables more rapid sound conduction. A third explanation is that the peribullary sinuses act as reflective boundaries which help channel sound, presumably from in front of the animal, toward the ears (Cranford et al., 2008b). These sinuses may change volume depending on the state of the animal and therefore affect acoustic delays from sound which enters behind the ear.

In order to further examine the shaded receiver model, latencies of AEP responses from the four stimulus locations were compared to the estimated sound velocity profile for the odontocete lower jaw acoustic fats (138 cm·ms^{-1}) (Blomberg and Jensen, 1976; Kobler et al., 1992). These jaw delays were, on average, 0.05 ms slower than those predicted for a straight line path from sound source to the location of shortest latency. However, rostrum tip stimulation reflected the smallest deviation with a mean delay of only 0.01 ms difference from a straight line path. The consistently higher values of measured delays vs. straight line predictions lend further support to the shaded receiver model. Delay differences for each stimulus location from a predicted straight line path support the theory of various sound channels, based on acoustic fat composition. Thus, we agree with previous work which suggests that odontocete localization and directionality depend on multiple factors in addition to simple shaded receiver model data (Branstetter and Mercado, 2006; Koopman et al., 2006; Cranford et al., 2008a).

Differences and similarities found between the sensitivities and latencies of beluga and dolphin AEP responses beg the question: Why? There were certainly some differences in experimental design between this and the Møhl et al. (1999) study, but procedures generally overlapped. Thus it seems likely there are differences in the way belugas and bottlenose dolphins receive sound, based in part on their head morphologies. How variation in head morphology affects hearing differences across a wide range of species, or even individuals within a species, requires greater attention. The directionality related differences also indicate that other auditory capabilities vary between species and extrapolating from only one species leaves limited conclusions. This underlines the importance of investigating hearing in the dozens of cetaceans not yet examined and stresses the need for caution regarding application of auditory characteristics to species for which we know relatively little, such as mysticetes and beaked whales. Because the variation in latency and sensitivity were found, this work supports the idea of a shaded receiver model in the beluga that includes variations based on hearing directionality and head morphology. Certainly, the hypothesis of a shaded receiver model for sound localization and much of odontocete directional hearing requires substantial additional data.

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