

Identifying Cetacean Hearing Impairment at Stranding Sites

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Abstract

While noise is now considered a marine hazard that can directly affect cetaceans and induce a stranding, no clinical approach has yet introduced the detection of a possible hearing loss at a stranding site as a necessary practice. This can be explained by the lack of time when facing vital decisions for the animal's welfare as well as the unavailability of reliable, lightweight, autonomous, and portable audiometry equipment. Herein, we correlate measured electrophysiological evidence of a permanent threshold shift (PTS) in a rehabilitated striped dolphin (*Stenella coeruleoalba*) that prevented its release, with the postmortem analysis of an abnormal dilatation of the central nervous system ventricles that prevented the correct acoustic reception of the animal. We further propose to follow a five-minute auditory evoked potential (AEP) standard protocol of hearing measurements in-air on cetaceans at a stranding site that includes the stimulation of auditory brainstem responses (ABRs) with a single 4- μ s broadband (> 150 kHz) pulse at three decreasing levels (129, 117, and 105 dB_{pp} re 1 μ Pa at 15 cm), which covers most of the cetaceans' known maximum acoustic sensitivity and allows the immediate sensing of an individual's hearing capability before any final clinical decision is taken.

Key Words: auditory evoked potentials, cetaceans, stranding, hearing impairment, striped dolphin, *Stenella coeruleoalba*, permanent threshold shift

Introduction

Acoustic trauma and other noise-related lesions have now been added to the list of potential causes of cetacean stranding (Richardson et al., 1995). Although natural noise may still be involved in the disorientation and death of individuals,

anthropogenic sources also have been shown to have the potential to adversely affect marine mammals in the form of noise-induced temporary (TTS) or permanent (PTS) threshold shifts (Ketten, 1995; Ridgway & Carder, 1997). While postmortem analyses have given some insight on the direct effect of sound exposure, which has been mainly expressed, but not exclusively, by lesions in the acoustic pathways of the studied specimens (Ketten, 1995, 1998; Degollada et al., 2003), the difficulty lies in detecting hearing impairment in live animals.

Studies on the effect of exposure to anthropogenic sound on wild animals through controlled exposure experiments (CEEs) have allowed some short-term behavioral observations of responses (André et al., 1997; Madsen et al., 2006; Noad et al., 2006), but the technology is not yet available to directly assess the CEE interaction with hearing processes.

The recent development of non-invasive electrophysiological techniques, in particular auditory evoked potentials (AEPs), to measure hearing in marine mammals offers a unique way to directly assess the hearing response of any individual cetacean after exposure to high amplitude sound (Dolphin, 2000; Supin et al., 2001; Supin & Popov, this issue). AEP methods only require minimal cooperation from the subject, and the responses can be obtained rapidly under very objective acceptance criteria.

In the laboratory, Au et al. (1999), Schlundt et al. (2000), Finneran et al. (2002, 2005), and Nachtigall et al. (2003, 2004) induced TTS in small odontocetes, principally captive bottlenose dolphins (*Tursiops truncatus*), and analyzed the resulting masking effects. They demonstrated a direct relationship between the sound source characteristics in terms of frequency, level, and exposure duration with the observed TTS and recovery times, thus considerably raising the level of understanding on the effects of noise. Nevertheless,

these studies have been performed on a very limited number of species and have obviously not crossed the boundary, for ethical and legal reasons, to induce PTSs. For the same obvious reasons, no animal has been sacrificed after inducing a TTS/PTS to correlate the electrophysiological findings with a postmortem analysis that would reveal some of the missing pieces of the cetacean hearing mechanism puzzle. Stranded individuals, because of the variety of the species and stranding conditions involved, represent a major source of information—not only to assess hearing sensitivity against noise exposure, but, most importantly, to complete the understanding of species-specific hearing, especially for species that have been little studied like beaked whales (Cook et al., 2006) or baleen whales.

A live stranding event for cetaceans is preceded by a natural or an anthropogenic (e.g., noise exposure) alteration of the animal(s)' normal functionality, which is often difficult to document—except on rare occasions when a direct cause-and-effect relationship has been established (Ketten, 1995; Balcomb & Claridge, 2001; Degollada et al., 2003; Fernández et al., 2003; Jepson et al., 2003). Electrophysiological measurements can still give information on the auditory status of the stranded individual, as well as a partial audiogram of the species. Recently, valuable data have been gathered on Risso's dolphins (*Grampus griseus*) (Nachtigall et al., 1995, 2005; Mooney et al., 2006) and striped dolphins (*Stenella coeruleoalba*) (André et al., 2003; Kastelein et al., 2003) that were until now two poorly documented species in terms of acoustic sensitivity.

Not all stranded individuals are suitable for transportation to research facilities where proper measurements can be performed, however, nor is rehabilitation always necessary when the stranding network team considers the animal healthy enough to be immediately released (Geraci & Lounsbury, 1993). In that case, there is very little time to deliberate, and if electrophysiological measurements have to be performed on site, they must be extremely fast and reliable enough to immediately support or oppose the veterinarian's decision.

The monitoring of hearing is not included in the stranding clinical protocol mainly because the AEP procedures not only require time, a non-available resource when facing vital choices for the animal's welfare, but most importantly, they could not be performed in the field.

Herein, we validate the inclusion of AEPs in clinical practice by describing the postmortem findings of a rehabilitated striped dolphin in relation to its electrophysiological hearing measurements, and further propose a standard protocol to be used

at stranding sites to instantaneously measure and diagnose hearing functionality in cetaceans.

Materials and Methods

Electrophysiological Measurements of Hearing vs Postmortem Findings

Subject—"Marisol" was a young female striped dolphin about 175 cm in length that stranded in August 2001 on the Mediterranean southern coast of Spain. After various failed release attempts on site, Marisol was taken for rehabilitation in the facilities of Mundomar in Benidorm, Alicante in Spain. The dolphin was hand-fed, gained weight, and periodic white and red blood counts showed no parasitic infestation nor serology problems. Its vital parameters remained at reasonable levels for the species. No ototoxic drugs were administered to the animal.

Stimuli—Prior to the scheduled release, electrophysiological measurements of hearing were performed (3 mo after the stranding and 4 mo prior to the dolphin's death). The stimuli used during this study were sinusoidally amplitude-modulated (SAM) tones, generated by a custom function generator and amplified by a B&K 2713 amplifier by activation of an individually calibrated piezoceramic transducer (B&K 8104 hydrophone). Their carrier frequency varied from 16 to 128 kHz. Amplitude-modulated tones were presented in bursts of 20-ms duration, modulation rate was 1,250 Hz, and modulation depth was 100%. Stimuli were presented at a rate of 20/s. The stimulating transducer was placed on the longitudinal axis of the dolphin's head at a distance of 1 m from the animal's head at a water depth of 20 cm. Stimulus intensity was specified in dB re 1 μ Pa of rms sound pressure. Marisol was held in a stretcher made with a sound transparent fabric and fixed at the centre of the pool in a 40- to 50-cm water column. This allowed the dolphin to remain under water while the dorsal part of the head and the blowhole stayed above the water surface.

Evoked Potential Collection—Evoked potentials were recorded using 1-cm disk electrodes inside 6-cm suction cups then secured on the dolphin's body surface. The active electrode was placed at the head just behind the blowhole. The reference electrode was placed on the back (both electrodes above the water surface). The recorded potentials were amplified within a passband of 5,000 Hz (flat frequency response up to 3,000 Hz, -3 dB at 5,000 Hz with 6 dB/oct slope beyond), digitized using an A/D converter, and averaged using a standard personal computer. The recording window was 30 ms long, thus allowing the recording of responses of up to 20-ms long amplitude-modulated bursts and

click trains. One thousand sweeps were averaged to collect one evoked-response record.

Postmortem Analysis

Marisol was found dead in the pool four months after the AEP experiments were conducted. A routine necropsy was performed immediately after death. The whole head was severed and injected with formalin through the internal carotids and common vertebral arteries. It was analyzed through an MRI (Siemens Somatom Plus 1T). During the dissection, suction of the cerebro-spinal fluid (CSF) was conducted by puncturing both brain hemispheres, replacing it with formalin. The brain was then extracted and sliced. After a close inspection of the auditory surrounding spaces, the ears were carefully extracted, isolating the tympanoperiotic complex. Fixation of the samples with formalin was performed by injection through the inner ear windows—that is, by dislocation of the stapes and gentle perfusion through the oval window. Together with the gross anatomy of the middle ear, the ears were processed for routine paraffin H/E staining histopathology after decalcification.

Recommended Standard Protocol to Monitor Hearing on Stranding Sites

OdiSEA: An Autonomous AEP Acquisition Unit—The Laboratory of Applied Bioacoustics developed an autonomous AEP acquisition system, OdiSEA (see Delory et al., this issue), that weighs less than 10 kg and can be carried by a single person, including electrodes, transducer, and cabling. The battery capacity allows three hours of operation, providing enough time to make a proper assessment of auditory function, including the measurement of the subject's complete audiogram.

OdiSEA consists of two battery-operated subsystems: (1) a PC laptop that runs a custom *LabView*[®] (National Instruments) application and drives a 6062E NI PCMCIA board. This subsystem generates the stimuli (generation of an arbitrary number of frequencies as high as 250 kHz), acquires the physiological response (the acquisition is synchronized with every stimulus onset), and processes the electrophysiological evoked response; (2) a battery-operated signal conditioning Peli[®] case preamplifies and filters the evoked response, and attenuates or amplifies the generated stimuli for proper piezoexcitation. The following biopotential filters are selectable from the front panel: a 50 Hz to 60 Hz selectable notch-filter, one high-pass filter with selectable 100 Hz and 500 Hz cutoff frequency, and one low-pass filter with 1 kHz and 10 kHz cutoff frequencies. Sensitivity can be selected for 80 or 100 dB gain in 20 dB steps.

To illustrate the proposed protocol, we tested the system on a 15-y-old bottlenose dolphin, Isaac,

in the facilities of Aquopolis, Tarragona, Spain (Parques Reunidos, S.A.). On stranding sites, the animal usually is found beached, lying on its belly, a position that inevitably leads to respiratory difficulties and to life-threatening lesions if not quickly removed (Geraci & Lounsbury, 1993). Under those in-air conditions, electrophysiological measures can still be performed with jawphones, a transducer embedded in a suction cup filled with medical transonic gel (Delory et al., 2006, this issue; Houser & Finneran, 2006). We simulated a stranding condition, lowering the pool water to its minimum level, with the animal almost touching the bottom. No specific attention was given to reduce background noise.

Rhythmically modulated sound stimuli are widely used in odontocete evoked-potential audiometry because they elicit a high-rate rhythmic sequence of ABRs (envelope-following response [EFR]) that can be more confidently extracted from background noise than a single ABR. This method requires a rather long time (about 90 min), however, which implies that the subject needs to be restrained in a comfortable position to assess the complete audiogram of the animal.

Popov & Supin (2001) showed that the ABR amplitude depends to a much larger level on the stimulus frequency bandwidth, rather than on spectrum level. The wider the stimulus frequency bandwidth, the larger the amount of neuronal assemblies that contribute to ABR generation. We stimulated the animal with a single 4- μ s broadband pulse (see Delory et al., this issue) at three different levels—105, 117, and 129 dB_{re} 1 μ Pa at 15 cm—that approximate a up-ramp of 24 dB with 12 dB steps, suitable to assessing the hearing response of the dolphin (Figure 1). To rapidly detect a serious hearing loss, we introduced an “end-ramp” protocol that could be species-specific. This protocol was applied at levels that are high enough to be above ambient noise (e.g., a breaking ocean wave), yet below the ABR-documented saturation that occurs at 130 dB and above (Popov & Supin, 1990a). Hence, this method contrasts with the usual hearing threshold estimation. In *T. truncatus*, “normal” hearing could be diagnosed when a clear response and positive gradient were observed at these three acoustic intensities, whereas “abnormal” hearing corresponded to absent or abnormally low responses (< 1 μ V) at these three levels and no saturation was reached for a complementary increase of 20 dB (here 149 dB re 1 μ Pa) if there was strong suspicion that the animal is hearing impaired. We believe this saturation-based, nonlinear protocol has the advantage of being less sensitive to animal size or age variations and, most importantly, is insensitive to the inevitable ambient noise.

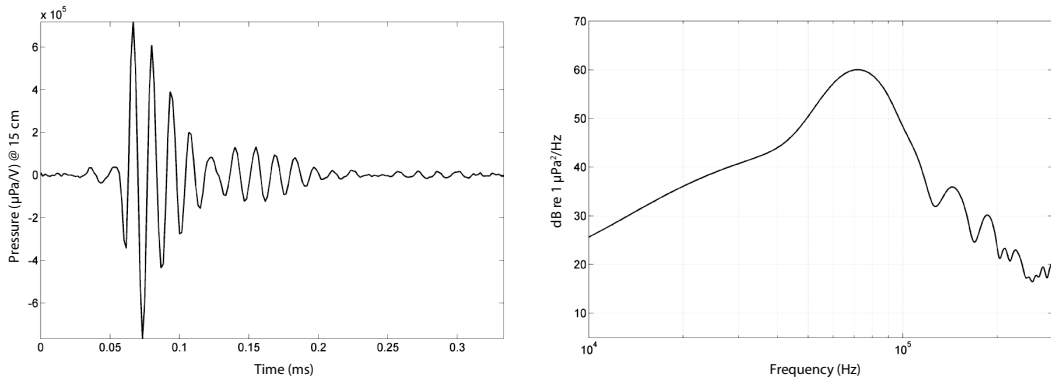


Figure 1. Waveform and spectrum of the transducer's acoustic response from the 4- μ s generated broadband click

While the best position of the transducer, through the use of jawphones, was determined by Møhl et al. (1999) to be at 10 cm below the eye, we wanted to verify the importance of the location of the acquisition electrode behind the blowhole as a function of the ABR amplitude. It can be difficult to accurately measure distances in stranding conditions, so we tested three positions: 10.0, 12.5, and 15.0 cm behind the blowhole.

Results

Electrophysiological Measurements of Hearing vs Postmortem Findings

The striped dolphin's thresholds were measured at frequencies from 16 kHz to 128 kHz, with $\frac{1}{2}$ -oct steps (at 90 kHz, the threshold was not determined because of strong contamination by electronic noise). The resulting audiogram is presented in Figure 2. The lowest threshold estimate (117 dB re 1 μ Pa) was obtained at a frequency of 45 kHz. Both at higher and lower frequencies, thresholds decreased to 132 dB re 1 μ Pa at 16 kHz and 131 dB re 1 μ Pa at 128 kHz.

When these results were compared with the behavioral audiogram values for the striped dolphin (Kastelein et al., 2003), where the maximum sensitivity, \sim 42 dB re 1 μ Pa, occurred at 64 kHz, it appeared that our subject animal responded with a 60 to 70 dB re 1 μ Pa less sensitive threshold values, indicating this animal could not hear any stimulus which did not go beyond abnormally high intensities (Figure 2). This dolphin probably found herself on the edge of the deafness threshold (André et al., 2003), and because of the consequent incompatibility of these results with a high survival probability, it was decided to cancel the imminent release. Interestingly, this hearing loss was the only apparent negative clinical parameter that came out of several weeks of analysis.

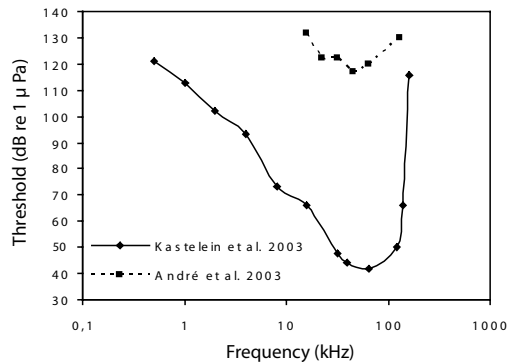


Figure 2. Striped dolphin (*Stenella coeruleoalba*) EFR threshold as a function of carrier frequency (audiogram); Marisol's audiogram (André et al., 2003) showed that this animal could not hear beyond abnormally high intensities.

Postmortem Analysis

The major postmortem finding was the dilatation of the cerebral ventricles containing a homogeneous and transparent liquid, which was compatible with lesions found in hydrocephaly. The cerebrospinal liquid showed no sign of infection nor alteration besides an abnormal volume. The expanded spaces were restricted to all the cerebral ventricles and their communicating channels. The plexus choroideus presented a normal shape and structure, indicating a possible normal function. Considering that the animal showed normal growth for its age, brain malformation as a cause of the stranding was discarded. The analysis indicated a physical obstruction of CSF flow and drainage of the ventricular system specifically at the fourth ventricle to explain the lesion.

The enlargement of the brain space produced a compression of the tissues, including the white and grey matters. This compression was especially important in the dorsal area affecting relevant areas, such as the temporal lobe known to be

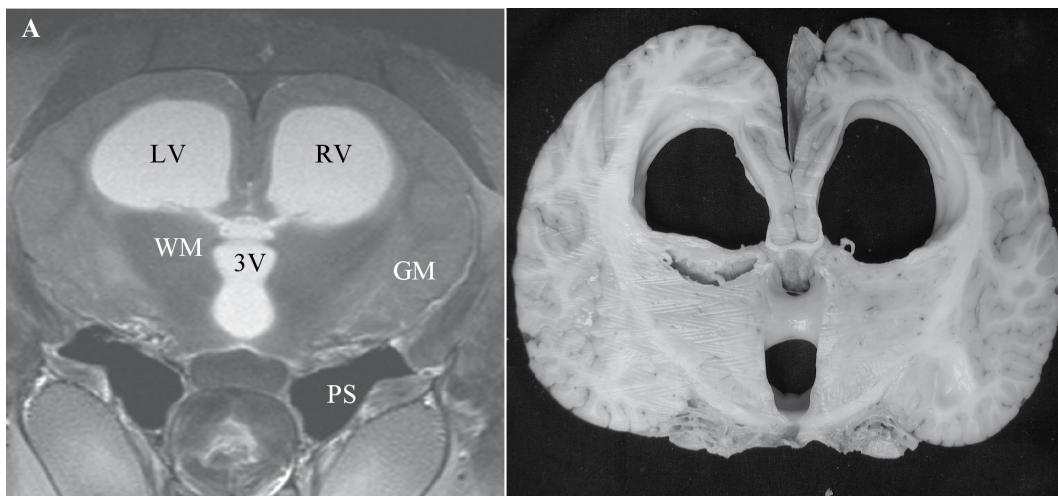


Figure 3. T2-weighted MRI of the cephalic region of Marisol (A) and the corresponding image of the brain slice fixed in formalin (B); RV: right ventricle, LV: left ventricle, 3V: third ventricle, WM: white matter, GM: grey matter, and PS: paraotic sinus. A dilatation of the cerebral ventricles is visible, compatible with lesions found in hydrocephaly.

the region of auditory processing (Morgane et al., 1986; Morgane & Glezer, 1990). In addition, the archipallium and other areas of the limbic system were also reduced, probably explaining the lack of reactions after basic stimuli. The histopathological analysis of the ear regions and inner ear structures showed neither lesions nor nerve volume alterations.

The PTS that was determined by the analysis of the electrophysiological measurements of Marisol offered a structural explanation, apparently independent from an acoustic source that could have induced lesions in the acoustic pathways. Although the AEP waveform cannot help discriminate the origin of a measured hearing impairment, in Marisol's case, we could perform a postmortem examination of the whole auditory acquisition chain, which shed light on where the alteration was located. To our knowledge, this is the first time that such a correlation (AEP results and postmortem findings) has been performed in cetaceans. Regardless of its pathological origin, the hearing impairment was not compatible with the release. At the time of the electrophysiological measurements, the dolphin's hydrocephaly had probably been developing since the stranding and continued until it died. If the electrophysiological measurements would have been conducted on day one of the stranding and just prior to death (7 mo time), the audiogram might have shown different threshold levels in correlation with the evolution of the ventricles' dilatation. This latter point is of particular relevance when deciding whether an audiogram performed on a stranded individual represents the species standard. This can only be

determined by systematically conducting electrophysiological measurements on a high number of specimens belonging to the same species, followed by a routine postmortem analysis of their acoustic pathways.

Standard Protocol to Monitor Hearing on Stranding Sites

Figure 4 shows the ABR of the bottlenose dolphin after stimulation with a 4-s broadband click (see Figure 1) at 1 V (129 dB_{pp} re 1 μPa at 15.0 cm) when positioning the acquisition electrode at three different distances behind the blowhole. Note that the response is correct at 10.0 and 12.5 cm, while it vanishes at 15.0 cm distance, confirming that

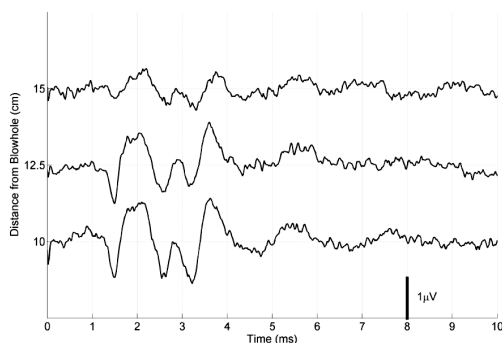


Figure 4. ABR of the bottlenose dolphin (*Tursiops truncatus*) after stimulation with a 4- μ s broadband click (see Figure 1) at 1 V (123 dB_{pp} re 1 μ Pa at 15.0 cm) at 10.0, 12.5, and 15.0 cm behind the blowhole; the response vanishes at 15.0 cm behind the blowhole.

for good AEP acquisition, the electrode must be placed immediately behind the blowhole at around 10.0 cm distance, allowing some positioning mistakes under pressure during a stranding.

Figure 5 shows the ABR of the bottlenose dolphin after stimulation with a 4- μ s broadband click (see Figure 1) at 2 V, 500 mV, and 125 mV. In humans, ABR waves are numerated based on latency following the delivery of the stimulus.

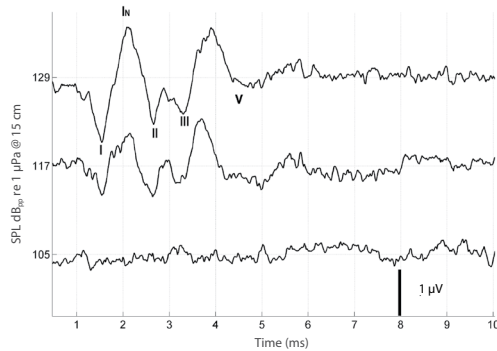


Figure 5. ABR of the bottlenose dolphin after stimulation with a 4- μ s broadband click (see Figure 1) at 2 V (129 dB_{pp} re 1 μ Pa at 15 cm), 500 mV (117 dB_{pp} re 1 μ Pa at 15 cm), and 125 mV (105 dB_{pp} re 1 μ Pa at 15 cm) at 10 cm behind the blowhole; the analogy with the typical human I, II, III, and V complex has not been proven to date. Note that AEP waveforms seem inverted compared to the typical response found in humans. This is probably due to positive and negative electrode swapping during the experiment. Also note that Peak V is, relative to the other peaks, not as high as could be expected in a human ABR response. To our knowledge, mapping of these peaks with functional locii has not been established in dolphins, hence human ABR peak indexing analogy should be used with caution.

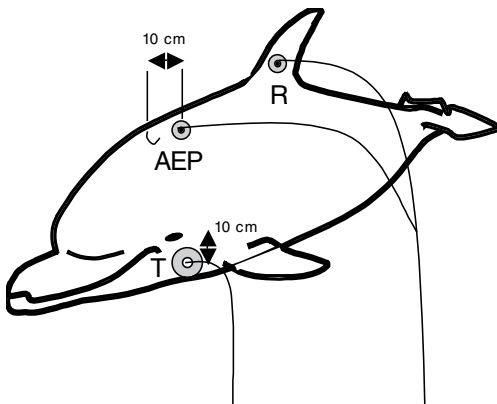


Figure 6. Acquisition (AEP), reference (R) electrodes, and transducer (T) positions for the AEP collection on stranding sites

The conventional method of identifying individual ABR waves is with roman numerals. At 2 V and 500 mV, what could be interpreted as Waves I, III, and V, considered to be the most clinically relevant components of the ABR, are clearly distinguished above noise and therefore consistent with a correct acquisition of the response from the set-up. These features of the response waveform that include the absolute latencies of the respective peaks of each wave, the time interval between peaks (most importantly, the I-III, I-V, and III-V inter-peak intervals), are most often used for clinical purposes to look for hearing impairment in humans (Jewett et al., 1971).

Given the fact that these ABRs reflect the stimulation of a portion of the organ of Corti, directly related to the bandwidth of the stimulus that leads to the synchronized discharge of neuronal units in the auditory system from the eighth nerve to the mid-brain, these results confirm that these simple measurements can be used to rapidly diagnose hearing impairment in dolphins.

The whole experiment, including the disposal of the electrodes and transducer on the animal, the synchronized ABR stimulation, and acquisition took less than 5 min to conduct. In other words, the acquisition, visualization, analysis, and storage of series of averaged ABRs elicited at three different acoustic levels allowed us to test whether the dolphin was hearing impaired with little doubt.

From Table 1, we can see that the exact same stimulus can be used to monitor hearing in the majority of odontocete species whose audiogram is known because it covers their maximum hearing sensitivity (50 to 100 kHz, -10 dB). For other species, including baleen whales, two additional transducers are needed to stimulate responses in the low- and mid-frequency bands.

In practice, some limitations and the resulting constraints are noteworthy. Considering the inter-species diversity in terms of stimulation bandwidth and the necessary sound pressure level (SPL) to reach response saturation, the proposed protocol may need to adapt to the species. Generally speaking, porpoise screening may require a transducer of higher resonant frequency than the one used for dolphins, while for larger whales, the transducer would inevitably need lower resonant frequencies and an amplifier able to deliver greater intensities (electrical). In the former case (i.e., porpoises), the transducer described in *OdiSEA* (Delory et al., this issue) coupled to the embedded 20-dB gain amplifier would be sufficient to properly stimulate the subject, while in the latter case (large whales, e.g., sperm whales and mysticetes), if not measured in calves, blubber thickness could be a limiting factor and could prevent collection of a measurable electrophysiological response.

Table 1. Known audiograms of odontocete species (from Morell et al., 2007)

Species	Frequency range (kHz)	Maximum sensitivity (kHz)	References
<i>Stenella coeruleoalba</i>	0.5-160.0 (B)	64.0 (B)	Kastelein et al., 2003
<i>Delphinus delphis</i>	11.0-152.0 (E)	60.0-70.0 (E)	Popov & Klishin, 1998
<i>Tursiops truncatus</i>	5.0-140.0 (E)	80.0 (E)	Popov & Supin, 1990b
	0.075-150.0 (B)	45.0 (B)	Johnson, 1967
<i>Phocoena phocoena</i>	10.0-160.0 (E)	30.0 and 125.0 (E)	Popov et al., 1986
	0.25-180.0 (B)	100.0-140.0 (B)	Kastelein et al., 2002
<i>Orcinus orca</i>	1.2-120.0 (E)	20.0 (E)	Szymanski et al., 1999
	4.0-120.0 (B)	12.0-20.0 (B)	Hall & Johnson, 1971
<i>Delphinapterus leucas</i>	~16.0-110.0 (E)	60.0-80.0 (E)	Popov & Supin, 1987; Klishin et al., 2000
	1.0-120.0 (B)	~30.0 (B)	White et al., 1978; Awbrey et al., 1988; Johnson, 1992
<i>Inia geoffrensis</i>	8.0-120.0 (E)	20.0-25.0 and 70.0-80.0 (E)	Popov & Supin, 1990c
	1.0-100.0 (B)	12.0-64.0 (B)	Jacobs & Hall, 1972
<i>Pseudorca crassidens</i>	2.0-115.0 (B)	16.0-64.0 (B)	Thomas et al., 1988
<i>Grampus griseus</i>	1.6-110.0 (B)	8.0-64.0 (B)	Nachtigall et al., 1995
<i>Lipotes vexillifer</i>	1.0-200.0 (B)	16.0-64.0 (B)	Wang et al., 1992
<i>Lagenorhynchus obliquidens</i>	0.10-140.0 (B)	64.0 (B)	Tremel et al., 1998
<i>Tursiops gilli</i>	2.0-135.0 (B)	30.0-80.0 (B)	Ljungblad et al., 1982
<i>Sotalia fluviatilis</i>	4.0-135.0 (B)	85.0 (B)	Sauerland & Dehnhardt, 1998

E: electrophysiological, and B: behavioural/psychophysical audiogram

Although our experiments took place in a noisy environment with no special care to reduce possible noise sources, we recommend that when the stranding condition and the size of the animal allows it, to position the subject on a neoprene foam carpet to limit the acoustic pathway from ground to skin. Aerial acoustic artifacts will most generally be negligible in view of the frequencies and impedances concerned.

Discussion

Because of their reliance on acoustics for both echolocation and communication tasks, testing the functionality of the auditory system of odontocetes is of special clinical importance before any final decision is taken such as to transport to rehabilitation facilities or to immediately release a stranded individual. The striped dolphin results showed that although the animal was found to be clinically stable after the stranding, it presented a serious hearing loss, incompatible with release, that could be first identified by AEP analysis and further corroborated by postmortem findings. Interestingly, the causes of the deafness were not directly found in the acoustic pathways, but in the presence of a dilatation of the cerebral ventricles, compatible with lesions found in hydrocephaly. These results underlined the necessity of introducing the monitoring of hearing on cetaceans at stranding sites. Since the survival rate of stranded cetaceans is very

low, electrophysiological measurements of hearing offer a unique opportunity to conduct a comparative analysis with postmortem findings. In addition, this dual routine analysis (AEP and immediate postmortem analysis) would not only allow the validation of audiograms from acoustically poorly studied species, but also improve our knowledge of stranding mechanisms.

Time often represents a limiting factor that may prevent the collection of complete audiograms at stranding sites. A compromise must be found when a fast decision is vital for the animal's welfare.

We propose a standard protocol that does not exclude the acquisition of an audiogram, if time allows, of in-air hearing measurements on stranding sites that includes (1) the use of a very light, autonomous, portable AEP acquisition unit; (2) the positioning of the acquisition electrode at 10 cm behind the blowhole and the embedded transducer at 10 cm below the eye; (3) the stimulation of a single pulse covering the maximum sensitivity of the majority of cetacean species, depending on the transducer response; and (4) the synchronized ABR acquisition. This protocol has been shown to be suitable to assess small odontocetes' hearing in less than five minutes, thus allowing its inclusion in clinical practice and the comparison with a high number of specimens.

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