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OdiSEA: An Autonomous Portable Auditory Screening Unit for Rapid Assessment of Hearing in Cetaceans

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Abstract

The screening of marine mammals' auditory capabilities is a vital and delicate diagnosis elaboration process. A self-configurable, compact, and portable battery-operated screening tool is now available, named OdiSEA, which enables the collection of species-related auditory characteristics and a rapid diagnosis of hearing impairment, both in controlled and field situations such as rehabilitation facilities and at stranding sites, respectively. Acoustic stimulation is achieved with a calibrated piezoelectric ceramic that transduces sound either through a gel-filled suction cup or, more conventionally, from a few meters distance to the subject in a pool. System portability and the integration of a wideband (> 150 kHz) auditory brainstem response (ABR) and multiple auditory steady-state response (multiple ASSR) evoked potentials system shortens diagnosis times significantly for both simple auditory tests and more detailed screening of auditory function. This unit should simplify and significantly accelerate the collection of audiograms in cetaceans.

Key Words: autonomous system, auditory evoked potentials, auditory steady-state response, envelope-following response

Introduction

The vital and specific nature of auditory function in aquatic mammals, added to the contextual diversity of its diagnosis elaboration process, led to the necessity of adapted assessment techniques and instruments. In cetaceans, like in human neonates (i.e., in noncooperative subjects), auditory evoked potentials (AEPs) are widely accepted as an *ad hoc* method to test hearing functionality in place of the more conventional behavioral protocol. Yet, in cetaceans, the diagnosis process tends to be prohibitively slow because of the frequent necessity of adapting an infrastructure to hold and secure cumbersome equipment, safely powering electrical appliances near a rehabilitation pool, and waiting and organizing work space for experts for, at times, several days. In emergency situations like strandings, on-site timely auditory tests to this point have not been technically feasible. Testing auditory function on stranding sites is the object of another paper in this issue; we point the reader to André et al. (this issue) for further details on this subject and the use of the solution described hereafter in stranding situations.

This article describes a self-configurable, autonomous, and portable unit for rapid auditory screening by means of evoked potentials. System self-configuration is facilitated by the possibility to load and save default or customized configuration files, avoiding the necessity to re-enter or modify stimulus parameters before and during the evaluation process. Designed to ease and accelerate the assessment of auditory function and the acquisition of audiograms in cetaceans, the unit reduces the overall diagnosis time substantially and, this probably is the most innovative aspect, can be used by non-experts with little training. It can be carried by one person and set to work in minutes. Gain in speed of high resolution audiogram acquisition can also be achieved using the preconfigured multiple auditory steady-state response (ASSR) protocol (Dolphin, 2000; John et al., 2001), which enables simultaneous testing at various frequencies. From a scientific standpoint, the unit is modular, customizable, and prepared for research on AEPs, allowing clicks, tone pips, and single or multiple amplitude and frequency-modulated waveform stimulations. The device calibration and specifications, as well as some of its capabilities, are described and illustrated with human and cetacean subjects. In order to keep this article concise and specific, we focus more on the innovative aspects of the device and, despite only having some of the configuration features tested so far (i.e., click-based auditory brainstem response

(ABR) and single ASSR), provide evidence of its overall potential, functionality, and safety.

Audiogram Assessment

The effectiveness and objectivity of AEPs in human auditory screening has motivated their use in marine mammals. Event-related electrical potentials in the cetacean brain were first reported by Bullock & Ridgway (1972). In two decades, this observation progressively opened the way to an objective and non-invasive method based on ABR, averaging and allowing for relatively fast and accurate assessment of the auditory sensitivity of odontocetes (Popov & Supin, 1990). Since then, confirmation of psychophysical measurements with electrophysiological responses was achieved for various odontocete species (see Dolphin, 2000, for a review). Most improvements in human electrophysiological auditory screening have been progressively tested and subsequently transferred to dolphin studies. One recent, noteworthy screening method that is also progressively being accepted is ASSR (also called envelope-following response [EFR]), which is a long-lasting response generated by modulated pure-tone stimuli, which result in better response level estimates. Use of ASSR makes low-frequency tests possible, and, most importantly, allows measuring multiple frequencies simultaneously with a single stimulus (Dolphin, 1997, 2000; John et al., 2001). ASSR could generate a reasonably densely populated dolphin audiogram in less than an hour.

Comparatively, the hardware improvements and portability found for human auditory screening instrumentation, such as commercial portable screeners for infants, have not been matched. The typical hardware setups in dolphin auditory screening still look like the one displayed in Figure 1. Although the efficiency of new ABR methods, such as ASSR, is unequivocal, especially for low-frequency audiograms, having to mount such an infrastructure evidently weakens the argument of greater processing speed that could be achieved by multiple ASSR. To complement this gain in speed and make the solution portable and functional in both controlled environments and at stranding sites, a drastic size reduction of the testing hardware must be achieved.

Several difficulties make this task challenging, among them odontocetes' hearing bandwidth is at least ten times wider than for humans, and transducing sound through bone, fat, or water may need the generation of very low-voltage (~mV), as well as greater voltage, signals (e.g., for waterborne stimuli) from the same low-voltage batteries. The unit ought to be splash-proof, light, and self-contained—both stimulation driving stages and biopotential preamplification should be secured and enclosed in the same container and controllable from an intuitive control panel. Control and acquisition software should allow broad diversity in terms of protocols but also be self-configurable for standard and routine tasks. The solution as a whole should also be modular enough and easily upgradable in order to be adapted to particular needs with little effort, and open to software improvements and new upcoming screening methods. We have addressed these needs and introduce the corresponding prototype, OdiSEA, which we will describe herein.

System

OdiSEA, also referred to below as "the unit," consists of two battery-operated subsystems:

1. A PC laptop runs a custom modular *Labview*® application and controls an A/D D/A 6062E National Instruments PCMCIA board. This subsystem generates stimuli up to 500 kS/s and simultaneously acquires and processes the preamplified electrophysiological response.
2. A battery-operated signal conditioning Peli® case preamplifies and filters the evoked response and attenuates or amplifies the generated stimuli for proper piezo-excitation.

Weighing less than 10 Kg, the whole system, which includes the unit, laptop computer, electrodes, transducer, and cabling, can be carried by one person. It has a total of three hours of continuous use autonomy, providing enough time to perform proper assessment of auditory functionality—for example, assessing the subject's third octave resolution audiogram from a few kHz upwards.

Materials and Methods

Stimulation and Acquisition Software

Two independent but synchronized functions are implemented. The stimulation part can be configured to generate clicks, tone pips, and ASSR (EFR) stimuli (i.e., AM and/or FM modulated carriers) and offers the possibility to generate an arbitrary number of carrier frequencies simultaneously by adding up to 10 waveforms up to a frequency of 250 kHz (so-called multiple ASSR). These tones can be mixed in different ways with independent levels, and AM or FM modulated with arbitrary parameters such as modulation depth and phase. Resulting $\mathrm{dB_{rms}}$ levels for each modulated carrier and the resulting waveform's spectrum, after the optional selection and application of a smoothing window, are then automatically calculated and displayed. A screenshot of the OdiSEA's waveform configuration window for a multiple ASSR stimulus is displayed in Figure 2. Of relevance here is the option to configure clicks and tone pips from this window, as well as from a preloaded and modifiable configuration file.

The stimulus waveform is generated as bursts of configurable duty-cycles, and the evoked

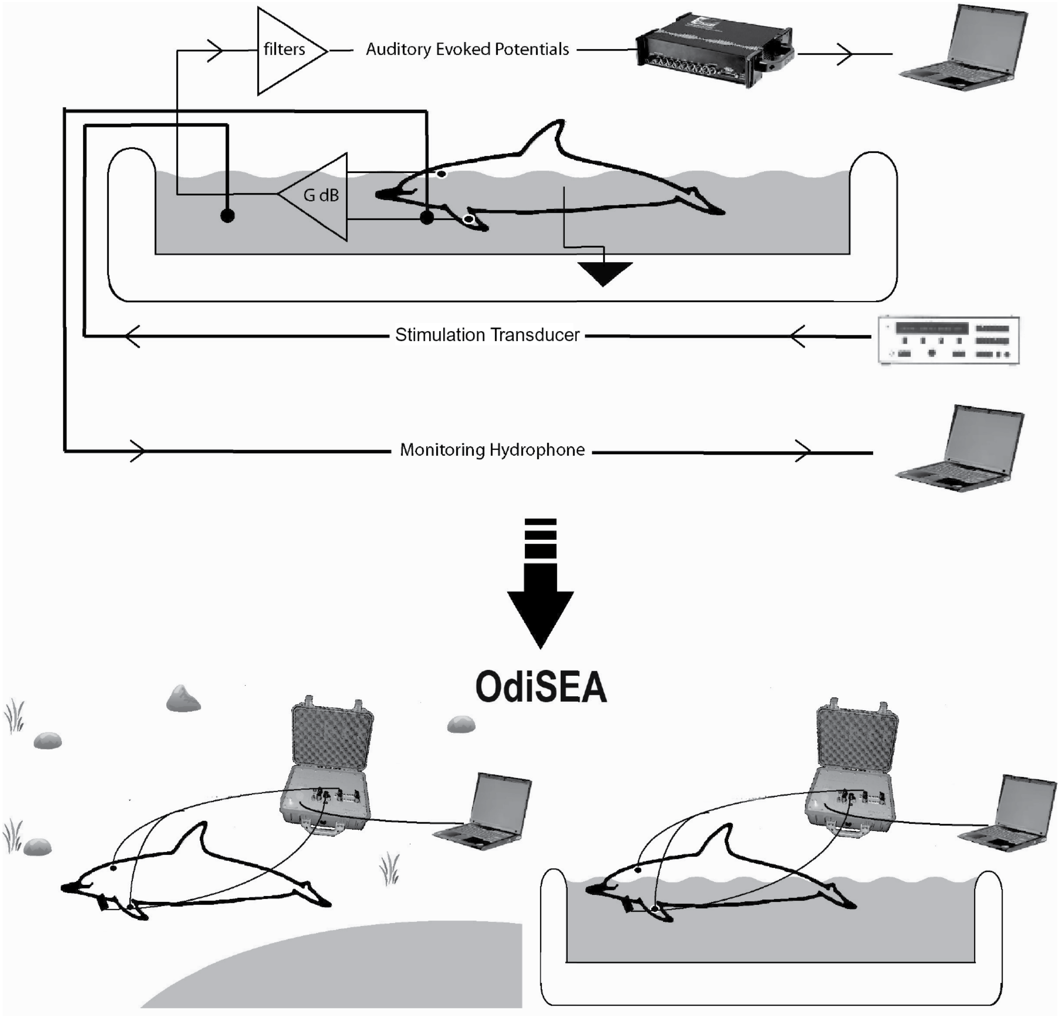


Figure 1. Conventional setup (top) and the OdiSEA unit used in emergency and controlled situations (bottom)

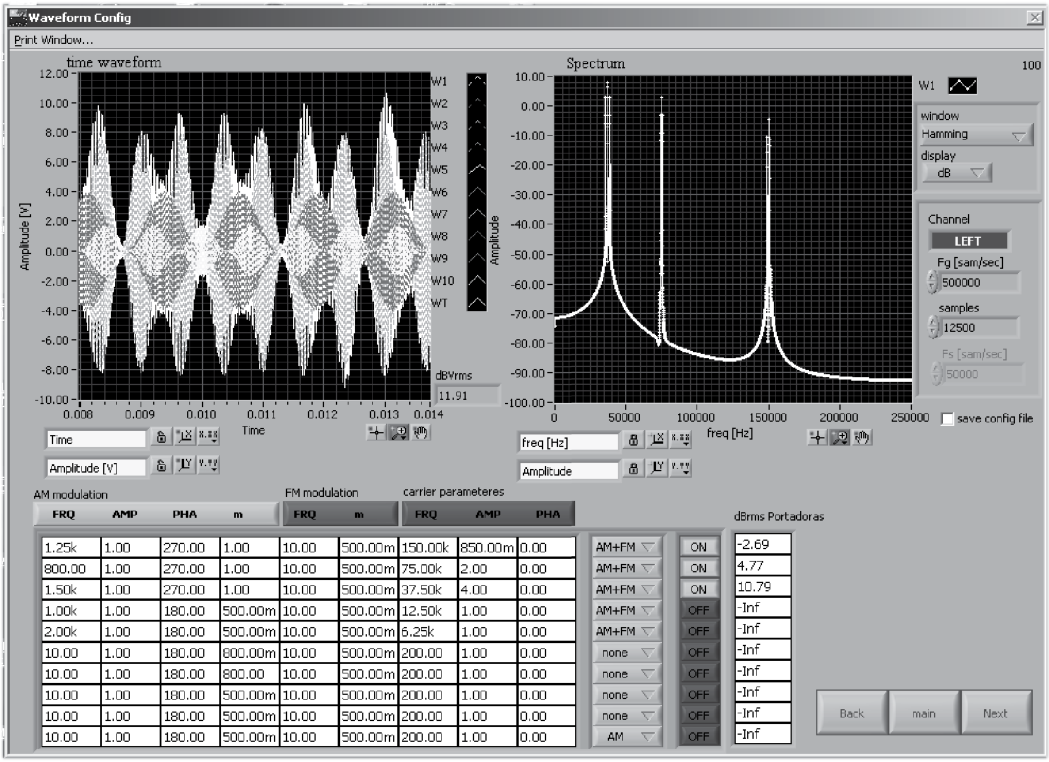
response acquisition is synchronized with every stimulus onset. Burst settings are configured in the next window (not shown here). While a given number of stimuli are being generated, the evoked response is acquired, building up coherently in a buffer by real-time averaging. The instantaneous and averaged responses are continuously displayed and updated on the computer screen during acquisition, providing better control and detection of artifacts, loose electrodes, or transducer, etc. The whole process is monitored by the acquisition panel (Figure 3). Stimulation ends with the automatic measurement of the response rms level.

Repeating the process with different stimulation levels and frequencies progressively generates the subject's audiogram (see André et al., 2003, for details). The software was written using *Labview® 7.1* (National Instruments). It was tested under *Microsoft Windows XP* on a Pentium III laptop with 256 MB RAM. A sample rate generation of 500 kS/s and simultaneous acquisition sampling rate of 50 kS/s could be achieved. Averaging of the evoked response could be performed and visualized in real-time, confirming the system performance for high-frequency standard AEP screening.

Signal Conditioning Subsystem

Stimulus amplification and AEP response preamplification are performed by a custom, medical-grade, battery-operated system which resides in a light-weight, shock-proof Peli® case. The prototype is displayed in Figure 4.

The transducer driver stage generates signals up to 200 kHz and 100 V output signal amplitude, suitable for both capacitive and resistive loads (except loudspeakers). Gain is selectable from -80 to 40 dB



dBVrms 11.91

window: Hamming

display: dB

Channel: LEFT

Fg [sam/sec]: 500000

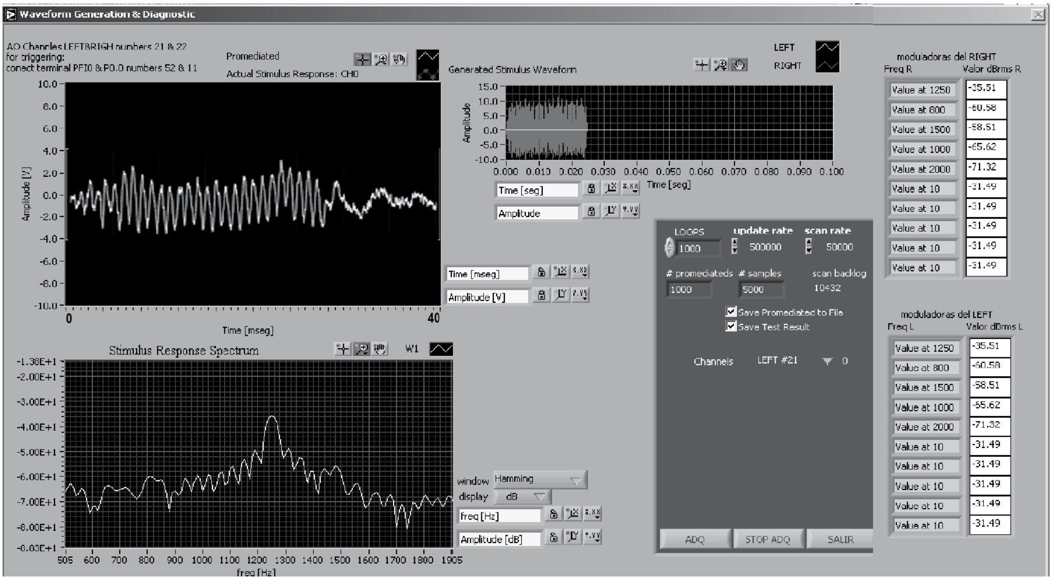
samples: 12500

Fs [sam/sec]: 50000

☐ save config file

AM modulation				FM modulation		carrier parameteres					dBrms Portadoras
FRQ	AMP	PHA	m	FRQ	m	FRQ	AMP	PHA			
1.25k	1.00	270.00	1.00	10.00	500.00m	150.00k	850.00m	0.00	AM+FM	ON	-2.69
800.00	1.00	270.00	1.00	10.00	500.00m	75.00k	2.00	0.00	AM+FM	ON	4.77
1.50k	1.00	270.00	1.00	10.00	500.00m	37.50k	4.00	0.00	AM+FM	ON	10.79
1.00k	1.00	180.00	500.00m	10.00	500.00m	12.50k	1.00	0.00	AM+FM	OFF	-Inf
2.00k	1.00	180.00	500.00m	10.00	500.00m	6.25k	1.00	0.00	AM+FM	OFF	-Inf
10.00	1.00	180.00	800.00m	10.00	500.00m	200.00	1.00	0.00	none	OFF	-Inf
10.00	1.00	180.00	800.00	10.00	500.00m	200.00	1.00	0.00	none	OFF	-Inf
10.00	1.00	180.00	500.00m	10.00	500.00m	200.00	1.00	0.00	none	OFF	-Inf
10.00	1.00	180.00	500.00m	10.00	500.00m	200.00	1.00	0.00	none	OFF	-Inf
10.00	1.00	180.00	500.00m	10.00	500.00m	200.00	1.00	0.00	AM	OFF	-Inf

Figure 2. Screenshot of the multiple ASSR stimulus waveform creation panel; in this example, all three pure tones are modulated in amplitude and frequency and properly mixed in order to generate a measurable ASSR. In a real situation, the level of each carrier will depend on the animal's expected response and the transducer response for each frequency. Carrier frequency intervals, levels as well as its respective modulation rates, must be carefully chosen to elicit a measurable response. All these parameters can be stored and recalled from a configuration file.



AO Channles LEFT&RIGH numbers 21 & 22
for triggering:
conect terminal PFI0 & P0.0 numbers 52 & 11

window: Hamming

display: dB

LOOPS: 1000

update rate: 500000

scan rate: 50000

promediateds: 1000

samples: 5000

scan backlog: 10432

☑ Save Promediated to File

☑ Save Test Result

Channels: LEFT #21 0

moduladoras del RIGHT

Freq R	Valor dBrms R
Value at 1250	-35.51
Value at 800	-60.58
Value at 1500	-58.51
Value at 1000	-65.62
Value at 2000	-71.32
Value at 10	-31.49
Value at 10	-31.49
Value at 10	-31.49
Value at 10	-31.49
Value at 10	-31.49

moduladoras del LEFT

Freq L	Valor dBrms L
Value at 1250	-35.51
Value at 800	-60.58
Value at 1500	-58.51
Value at 1000	-65.62
Value at 2000	-71.32
Value at 10	-31.49
Value at 10	-31.49
Value at 10	-31.49
Value at 10	-31.49
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Figure 3. Monitoring panel of the evoked response acquisition, with waveform spectrum and RMS levels at the modulation frequencies for each tone under test (right)

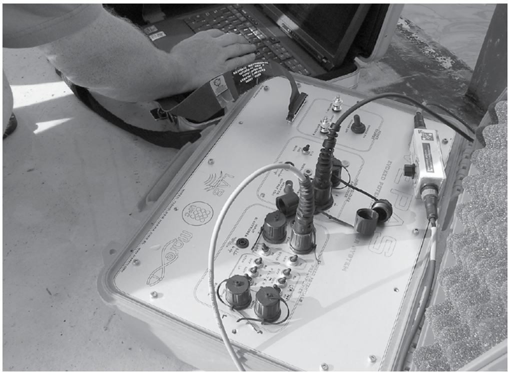

Figure 4. Signal conditioning case, with control and connection panels of both transducer excitation (top) and evoked potential preamplification and conditioning (bottom); a manual capacitive attenuator can be seen on the right in line with the transducer cable. This attenuator is used to reach the lowest levels (-80 dBV).

in 20 dB steps. The power supply is provided by a power-efficient and low-noise step-up 12 to 100 V DC-DC converter. The relatively higher voltage driving capacity found in this unit will make auditory screening of hearing impaired animals possible, as was shown necessary in André et al. (2003).

The preamplifier stage is specifically designed for electrophysiological signal conditioning and is customized for dolphin AEPs. It has very low noise characteristics, and signal high-order filtering options are numerous considering the system size: one high-pass filter with selectable 100 Hz and 500 Hz cutoff frequencies and one low-pass filter with 1 kHz and 10 kHz cutoff frequencies. A 50 Hz (that can be changed to 60 Hz) notch-filter and sensitivity change from 100 to 80 dB can be selected from the front panel.

The greatest concerns in integrating possibly high and low voltage signals into the same portable system were safety and interferences. Signal leakage from the stimulation stage that could alter the EEG signal was overcome by complete physical separation. Both stages are also supplied by two independent batteries. With respect to subject safety, the EEG preamplifier complies with EN 60601 medical regulations. Also, the possibility to send voltages higher than 10 V to the acoustic transducer is only enabled when the required amplification is manually switched on. The transducer is embedded in various layers of solid epoxy, providing proper and reliable electrical insulation. Only switching on the higher voltage transduction stage when necessary is an effortless way to further increase the subject's electrical safety. The user could also decide to use sounds of higher amplitude only for the standard 1- to 2-m distance waterborne measurements, hence avoiding physical contact with the transducer.

Added to physical and electrical partitioning, a Faraday cage shields the EEG stage from stimulation signal interference. The case's aluminum front panel can be put to ground via a 4x25 mm connector ("banana" connector); the same applies to the EEG preamplifier stage common input. These grounding options help shield the unit from external interferences and can set the animal to ground when found necessary through the ground electrode.

Transducer and Electrodes

As previously confirmed through AEP threshold measurements with jaw-phone stimulation as compared to behavioral thresholds, jaw-phones can be used for hearing threshold estimation, with acceptable differences (Houser & Finneran, 2006). Likewise, the OdiSEA suction-cup transducer is a piezoceramic embedded in multilayered epoxy, wrapped in closed-cell neoprene rubber tape and held on the animal's lower jaw via a soft polyurethane FESTO suction cup that we generally fill with hypo-allergenic, medical-grade ultrasound gel. This transducer's physical conditioning, along with its use in a non-free field, required proper calibration, which we performed at the back of our laboratory at a 2-m distance from the harbour's quay in 3-m water depth. For this calibration, see Brill et al. (2001). For a similar procedure, we used a preamplified, individually and recently calibrated Brüel & Kjaer 8101 hydrophone, an Agilent 33120A waveform generator, a 1 GS/s Tektronix digital oscilloscope, and an IOtech Wavebook 516™ 1 MHz sampling rate acquisition system. Frequency and impulse responses of the full stimulation stage, with and without amplifier or attenuator, were measured with the suction cup transducer facing the B&K 8101 hydrophone at a 25 cm distance (Figure 5). Spherical propagation correction was applied when necessary for 15 cm distance-level estimation as in Houser & Finneran (2006). Calibration results include pure tone response (Figure 6) and impulse (i.e., a broadband click) response, the waveform and spectrum of which are displayed in Figures 7 and 8, showing the actual acoustic energy radiated as a result of sending a 4 μs square wave directly to the transducer with no amplification, nor attenuation.

OdiSEA's reusable electrodes (Figure 9) are embedded in flat and firm polyurethane FESTO suction cups and connected to the case via a 3-m shielded coaxial cable that can be extended to 8 m by an IP68 waterproof extension. When using the extension, the connections can stand 1-m water depth. Electrode disks are Invivometric 24 x 1 mm Ag-AgCl disks. The disks were embedded within the suction cups using epoxy resin. OdiSEA was tested with these electrodes, using standard electro-gel, the 5-m cable with no extension, and compared with medical-grade commercial pre-gelled disposable Lessa® electrodes

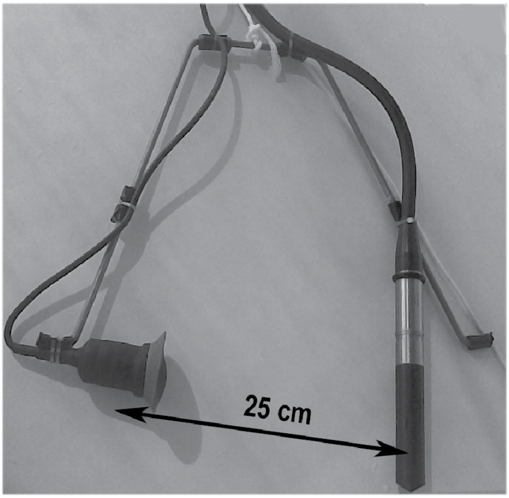

Figure 5. OdiSEA's suction-cup transducer and B&K 8101 hydrophone calibration setup; acoustic insulation pads at each cable and transducer attachment point reduce acoustic leakage through the frame.

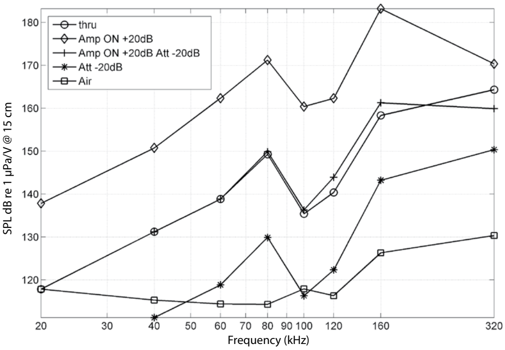

Figure 6. Acoustic transduction frequency response at 15 cm from the suction-cup transducer, acquired using pure tones as input to direct (through), amplified, attenuated, and both amplified and attenuated stimulation stages; the "Air" curve results from transducing sound through air in order to measure an upper bound of a possible acoustic leakage through the metallic frame seen in Figure 5.

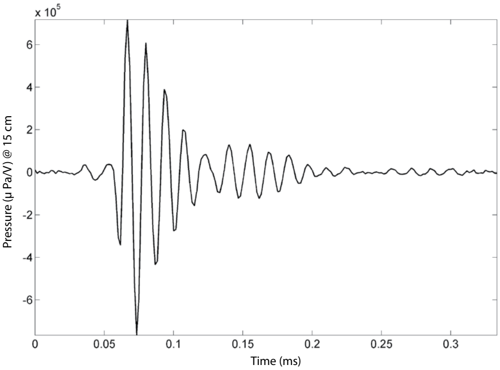

Figure 7. Suction-cup measured acoustic impulse response, from a 4 μs square pulse; peak-to-peak maximum corresponds to 123 dB re 1 μPa/V @ 15 cm.

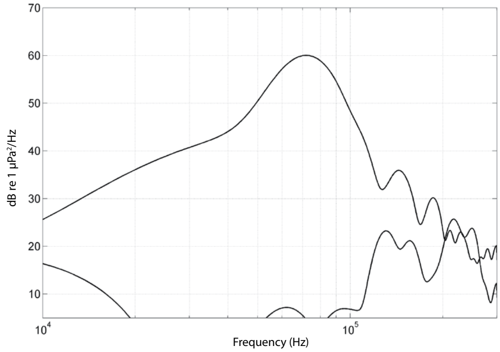

Figure 8. Estimated power spectral density of the transducer acoustic impulse response at 15 cm distance (waveform shown in Figure 7), showing a -10 dB 50 kHz bandwidth centered on 75 kHz; bottom curve shows the contribution of background noise to the measurement by repeating the calibration with no signal.

and a 1-m standard thin cable on a human adult subject, using headphone stimulation. The results are presented in Figure 10, showing the efficiency of the OdiSEA electrodes, despite a much longer cable. As expected, the fine sensitivity is believed to result from the electrode's larger diameter, which probably compensates signal loss through the cable.

Results

System Validation in Cetaceans

OdiSEA was tested on a 15-y-old bottlenose dolphin (*Tursiops truncatus*), Isaac, in the facilities of Aquopolis, Tarragona, Spain (Parques Reunidos, S.A.).

The objective was not only to test the correct acquisition of various types of AEPs, but also to check whether the whole system's installation was simple, reliable, and fast. Test conditions were probably comfortable compared to a stranding situation as the animal would not show adverse reactions when held quiet by the curator for 15 min in a row, repeatedly receiving a great diversity of acoustic stimuli from broadband clicks to amplitude modulated tones. The transducer was positioned 10 cm down from the right eye, an area of minimal sensorial variability (Møhl et al., 1999). Total time for OdiSEA's installation, from our time of arrival on the working platform to the time we were ready to send stimuli, was generally less than 5 min after a few trials. Then, in less than 5 min, we were able to acquire, visually analyze, and store

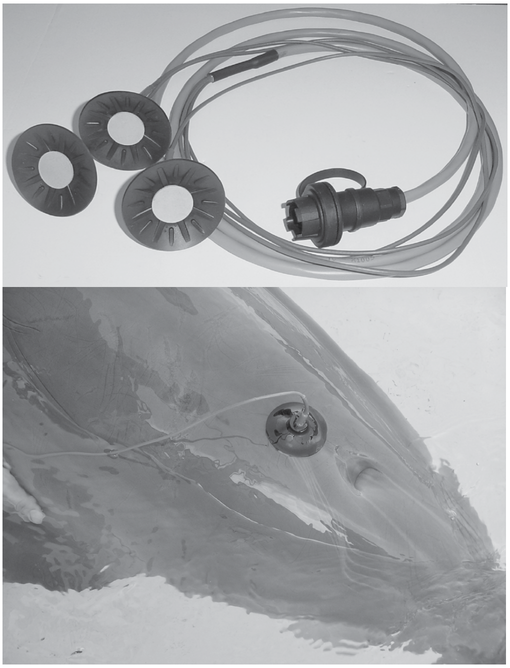

Figure 9. OdiSEA's electrodes with 3-m cable and IP68 connector (top); signal electrode positioned 10 cm behind Isaac's blowhole (bottom).

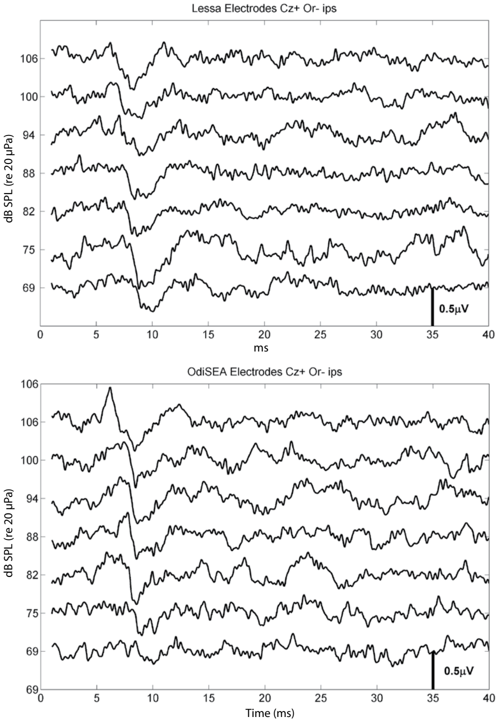

Figure 10. ABR response in a human adult subject using OdiSEA's hardware and software with commercial medical-grade pre-gelled and pre-wired Lessa® electrodes (top) and OdiSEA's custom-made suction-cup embedded electrodes with 3-m cable (bottom); in both figures, the V-wave varying latency is typical of human ABR responses at different stimulation levels.

5 to 10 series of averaged ABR responses elicited at different acoustic levels. This means that in less than 10 min, OdiSEA allowed us to test whether the dolphin was hearing impaired with little doubt on our results. ABR responses at different stimulation levels, showing typical patterns (Popov et al., 2001), are plotted in Figure 11. Though we have not yet had the opportunity to acquire a complete audiogram, we could acquire some ASSR responses using amplitude-modulated carriers. It generally took 1 min to acquire one ASSR properly, averaging 1,000 acquisitions of 25-ms bursts, with a 50-ms repetition period. Response to ASSR stimulation at 80 kHz is shown in Figure 11. At the time of submission of the manuscript, ASSR and multiple ASSR still needed further tests in order to be validated, but the preliminary tests presented here show the device functionality for this method.

Discussion

While currently in its final test stage, we hope to be able to make this instrument available to the scientific community and cetacean rescue organizations soon. Some hardware improvements are planned in the coming months from the time we submitted the manuscript, which will mainly consist of the addition of a sound level meter so as to know from the same unit the background noise the animal is exposed to at the time of AEP collection. This will help discard possible apparent deafness because of high background noise and reduce the probability of a wrong diagnosis in emergency situations. Still, for in-air measurements, this probability might be very low as stimulation frequencies are rather high compared to commonly found ambient noise spectra in the air.

Steady-state responses with OdiSEA have not been thoroughly tested yet as data were only acquired for a few frequencies on one subject. At times, we observed artifacts that could possibly be a consequence of electromagnetic leakage from the stimulus propagating through the subject's head. Though such leakage can be easily discarded visually via the observation of a typical few milliseconds delay between stimulus and the evoked response, we hope to be able to detect the physical source of the problem and make the system more robust to interferences. We have observed that sending alternatively positive and negative stimuli to average off or rule out the electromagnetic artifact would not always prevent it from happening as noticed in Picton & John (2004). This issue is currently under investigation.

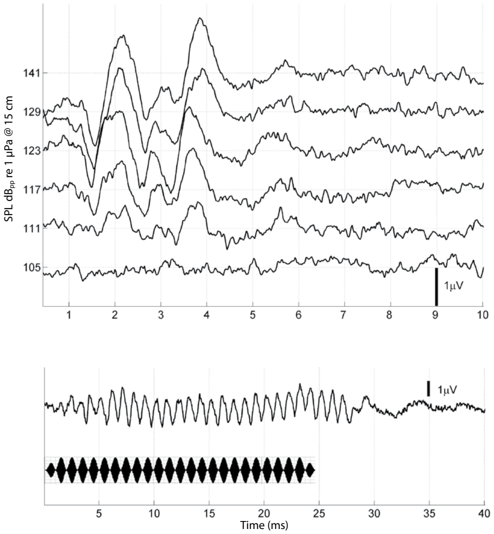

Figure 11. ABR responses to 4 µs click stimuli on bottlenose dolphin, obtained with OdiSEA with suction-cup transducer 10 cm below the right eye, electrodes positioned 10 cm behind the blowhole and on right pectoral fin (top); ground electrode was in contact with water and case was connected to ground through the electrode. ASSR, with same transducer-electrode configuration as in top figure, using 80 kHz tone amplitude-modulated at 1kHz frequency (bottom); stimulus is shown below that line.

The unit was designed for cetaceans, but it can be used for other marine or terrestrial mammals as well. The difficulties raised by screening cetaceans have actually driven us to design a system of greater constraints (greater bandwidth, greater speed, etc.), which can drive a piezoelectric transducer (conventional headphones have been tested, too, as seen in the human tests in the previous sections). Consequently, most specifications in OdiSEA outperform the conventional medical auditory screening systems found for humans, thus it can be used for less technologically demanding aerial ears, too.

The presented toolset, OdiSEA, is autonomous, portable, self-contained, self-configurable, and integrates numerous ways to elicit AEPs in cetaceans, whether by means of classical but rapidly acquired ABRs to broadband clicks (less than 5 min) or more precise audiogram estimation via modulated waveforms (e.g., tone pips, single and multiple frequency steady-state stimuli). We are confident that these unprecedented features will prove efficient and useful for conventional or more urgent marine mammal hearing assessment, both in rehabilitation centers and at stranding sites.

Acknowledgments

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