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# Electrophysiological Measurements of Hearing in Marine Mammals

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Cetaceans possess remarkable auditory capabilities, both in the frequency and time domains. Their extensive use of sounds is particularly important during biosonar processes but also in communication. Their wide broadband frequency sensitivity, with hearing ranging from perhaps less than 20 Hz in baleen whales to greater than 160 kHz in many delphinid odontocetes, seems to also be integrated with detailed temporal resolution.

Until very recently, although the auditory system of cetaceans has attracted considerable interest, hearing capabilities of cetaceans have been studied for a limited number of species (Yuen et al. 2005). Because legal and ethical considerations prevent invasive physiological approaches to studying hearing in cetaceans, such as those that have been conducted with other species like bats, most of the available data concerning hearing sensitivity (audiograms) in cetaceans were obtained through psychophysical and behavioural methods, which required the cooperation of the animal through precise training: the bottlenose dolphin (*Tursiops truncatus*) (Johnson, 1967), harbour porpoise (*Phocoena phocoena*) (Andersen, 1970), killer whale (*Orcinus orca*) (Hall & Johnson, 1971; Symanski et al., 1999), Amazon river dolphin (*Inia geoffrensis*) (Jacobs & Hall, 1972), beluga whale (*Delphinapterus leucas*) (White et al., 1978; Awbrey et al., 1988; Johnson, 1992), false killer whale (*Pseudorca crassidens*) (Thomas et al., 1988), Risso's dolphin (*Grampus griseus*) (Nachtigall et al., 1995), Chinese river dolphin (*Lipotes vexillifer*) (Wang et al., 1992), and Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) (Tremel et al., 1998).

Early studies (e.g., Popov et al., 1986) showed that electrophysiological methods, especially the analysis of auditory evoked potentials (AEPs), were highly appropriate for use in cetaceans and other marine mammal species. AEPs are physiological recordings of electrical pulses generated by neural activity within the brain in response to acoustic stimuli. AEPs can be obtained in a non-invasive way through electrodes attached to the scalp surface of many species. Recently, this technique has allowed researchers to confirm psychophysical data and begin to "fill in the gaps" in the cetacean audiogram database, at least for odontocetes.

Together with the development of the technique, the importance of precisely determining the hearing capabilities of marine mammals has increased in the last 10 years. It is now becoming clear that human-made noise, at different intensity levels, can negatively affect marine mammals, including avoidance reactions, collisions with ships, mass stranding, and death. The current scientific knowledge on the effects of noise on marine mammals and their habitat is still insufficient to understand the relationships of frequencies, intensities, and duration of exposures to various types of noise and the possibility of producing damage. Certainly, much more research is required.

In light of recent cetacean mortality events, the Council of the European Cetacean Society (www.europeancetaceansociety.eu/ecs) wrote a statement concluding its 17th Annual Conference on Marine Mammals and Sound that considers the following issues:

- Research on the effects on human-made noise on marine mammals is urgently needed and must be conducted to the highest standards of science and public credibility, avoiding political concerns and potential conflicts of interest.
- Non-invasive mitigation measures to protect cetacean hearing must be developed and implemented as soon as possible.
- The use of powerful underwater noise sources should be limited until their short- and long-term effects on marine mammals are better understood, and they should not be used in areas important for cetaceans such as known breeding and feeding areas.
- Legislative instruments must be developed that help implement both national, European, and international policies on marine noise pollution.

These new elements request a dynamic analysis of the situation that must go through the development and implementation of new techniques and technologies without slowing down human interests and needs nor compromising the conservation of the marine habitat.

AEPs constitute a promising technique to assess hearing thresholds quickly in many marine mammal species. In March 2006, an international workshop was organised by Michel André and Paul Nachtigall and hosted by the European Cetacean Society during its 20th Conference in Gdynia, Poland, which brought together experts in the field of bioacoustics and hearing. They presented a summary of the "state-of- the-art" of AEP studies on cetaceans and non-cetacean species as well as provided directions for future research. This special issue of *Aquatic Mammals* represents the output of this workshop.

**Nachtigall et al.** offer an overview of the current knowledge on how AEPs and acoustic brainstem responses (ABRs) have been used to measure hearing while an odontocete is actively echolocating. This technique of measuring the animal's ability to hear its own outgoing signals, as well as the returning echoes, allows experimenters to develop a new understanding of the processes underlying echolocation.

**Supin & Popov** demonstrated that the efficiency of the AEP method can be markedly increased in odontocetes by the use of (1) stimulus parameters providing maximal AEP amplitude and (2) methods of better extraction of AEP from background noise. They proposed using an effective stimulus, a train of short tone pips that allows the same analysis technique as sinusoidally amplitude-modulated (SAM) stimulus but provides a much higher AEP amplitude and a very effective median-based extraction when the noise is not stationary and includes short but big spikes or bursts.

Most of the knowledge of hearing in cetaceans comes from research conducted with small odontocetes, particularly the bottlenose dolphin. **Popov et al.** conducted AEP analysis on a representative number of animals to document audiogram variability. Fourteen subjects, 11 males and three females, were investigated. All the subjects appeared to present qualitatively similar audiograms except one. The averaged audiogram featured the best sensitivity (the threshold below 50 dB re 1 µPa) at 45 kHz. Thresholds rose slowly to lower frequencies (up to 65 dB at 8 kHz) and steeply at higher frequencies (up to 97 dB at 152 kHz). Based on these data, the authors also suggest an analytical formula for a standard audiogram.

**Hernandez et al.** studied middle- and long-latency AEPs to detect the discrimination ability of an individual. To investigate the characteristics of evoked responses resulting from the "oddball paradigm," the authors recorded AEPs from two bottlenose dolphins. The results demonstrate sensory gating, either habituating to a repeated stimulus (gating-out) and/or dishabituating to a novel stimulus (gating-in). The presence of one or both of these responses suggests that the P50 response to oddball stimuli has the potential to indicate discrimination of a particular set of auditory stimuli.

**Finneran et al.** measured auditory steady-state responses (ASSRs) in a bottlenose dolphin and used them to illustrate objective techniques to determine the presence or absence of a response. Two frequency-domain techniques were used to assess the presence or absence of a response: (1) the F-test compares the evoked potential power at a single frequency (the amplitude-modulation frequency) to the noise power averaged over adjacent frequencies, and (2) magnitude-squared coherence (MSC) is a ratio of the signal power at a single frequency to the signal-plus-noise power and reflects the degree to which the system output is determined by the input. The authors found that both techniques provided identical results and concluded that evoked potential thresholds based on the lowest detected response compared favorably to behavioral thresholds obtained on the same species in the same environment.

Another cetacean species of major conservation interest is the harbour porpoise (*Phocoena phoceona*). **Lucke et al.** conducted a study on a harbour porpoise to measure the audible range of wind turbine-related sound emissions and their potential masking effect on the acoustic perception of the animal by measuring AEPs. AEPs were evoked with two types of acoustic stimuli: (1) click-type signals and (2) amplitude-modulated signals. The resulting data showed a masking effect of the simulated wind turbine sound at a level of 128 dB re 1µPa at 0.7, 1.0, and 2.0 kHz. The authors concluded that the potential masking effect from wind turbines would be limited to short ranges in the open sea, but they also warned that all estimates are based on existing turbine types and do not take into account future developments of larger and potentially noisier turbine types.

**Beedholm & Miller** described how a stationary harbor porpoise altered the rate and amplitude of its echolocation clicks when presented with an artificial target at a fixed delay. The animal spontaneously changed the click rate in such a way that the emitted level (in dB, arbitrary reference) of a click decreased as the inter-click interval (ICI) decreased according to a 14.5 log (ICI) function. This same relationship was found when the animal swam towards a target (a fish). The porpoise reduced the amplitude of clicks as it approached the target at a rate of -14 to -17 log r. The authors suggested that the combined results indicate an incomplete automatic gain control (AGC) working on the transmitter side that might be explained by constraints in the sound-production apparatus that couple the sound amplitude to the click rate.

The Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) was studied by Tremel et al. (1998) using behavioural techniques that measured the audiogram of this little-known species in terms of auditory sensitivity. Herein, **Au et al.** present measurements of underwater hearing thresholds of the same dolphin at John G. Shedd Aquarium in a broadband masked environment using ABR methods. The ABR waveforms were slightly different than for other odontocetes, having seven to eight waves present. The peaks in the Fourier transform of the ABR waveform occurred at 650 and 1,200 Hz, very similar to the 600 to 650 and 1,100 to 1,200 Hz for *Tursiops truncatus.* Masked ABR thresholds expressed in peak-to-peak values were between 24 and 41 dB above the peak-to-peak values of the masking noise.

Because data on acoustic sensitivity are needed from a wide number of marine mammal species to assess the possible effects of noise pollution, the access to stranded individuals will certainly become a major source of information—not only on species-specific characteristics but also on the functionality of the auditory systems at stranding sites. **Delory et al.** present a self-configurable, compact, and portable battery-operated screening apparatus, which enables the collection of species-related auditory characteristics and a rapid diagnosis of hearing impairment, both in controlled environments such as rehabilitation facilities and in field situations like stranding sites. Acoustic stimulation is achieved with a calibrated piezoelectric ceramic element 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) ABR and a multiple auditory steady-state response (multiple ASSR) evoked potentials system shortens the diagnosis times significantly for both simple auditory tests and more detailed screening of auditory function.

**Taylor et al.** also created a portable system that is capable of measuring the hearing thresholds of marine mammals in field conditions. This system consists of multiple individual components, independently purchased or assembled. The major component of the system is a standard laptop computer with custom-written *LabView*® software able to both generate outgoing signals and acquire the corresponding brainwave measurements in response to those outgoing signals. The system is still in an ongoing state of improvement and optimization with the goal of having a final system that could be used in almost all field conditions.

**André et al.** correlated the measured electrophysiological evidence of a permanent threshold shift (PTS) in a rehabilitated striped dolphin (*Stenella coeruleoalba*), which prevented its release with the postmortem analysis of an abnormal dilatation of the central nervous system ventricles that canceled the correct acoustic reception of the animal. The authors further proposed to follow a 5-min AEP standard protocol of in-air hearing measurements on stranding sites that includes the stimulation of ABRs with a single 4-μs broadband (> 150 kHz) pulse at three decreasing levels (129, 117, and 105 dB pp re 1μPa at 15 cm), which covers most of the known cetacean maximum acoustic sensitivity and allows the immediate sensing of the individual hearing capability before any final clinical decision is taken.

**Houser et al.** investigated AEPs in northern elephant seals to characterize the responses elicited by different acoustic stimulus types, examine temporal resolving capabilities, and evaluate the potential for using evoked responses to estimate hearing sensitivity. Clicks and tone pips were presented to individual seals to characterize evoked responses to broad- and narrowband stimuli. Tone pip trains and SAM tones were used to determine modulation rate transfer functions (MRTF) of the auditory system and to determine if the magnitude of the envelope-following response (EFR) relative to the stimulus level can be used to estimate hearing thresholds. Click-evoked responses were characterized by three early positive peaks (~2.6, 4.4, and 6.1 ms) and a dominant negative peak at 7.2 ms and had average amplitudes of 264 nV (pk-pk) for a corresponding stimulus level of 126 dB re 20 μPa (pk-pk). Both the rate following response (RFR) and EFR amplitudes were maximal when the stimulus repetition rate or the amplitude modulation rate, respectively, were < 100 Hz. EFR amplitudes at the rate of amplitude modulation tracked near linearly with stimulus level. Thresholds for a 4 kHz SAM tone were estimated to be 42 dB re 20 μPa.

Based on a recent electrophysiological investigation of manatee (*Trichechus manatus*) hearing that showed a better temporal resolution than expected (Mann et al., 2005) that led to speculation that enhanced temporal processing capabilities are adaptive for underwater sound localization, **Mulsow & Reichmuth** measured evoked responses from three male and two female California sea lions, a harbor seal, and a northern elephant seal to determine how well the auditory systems of these amphibious mammals resolved rhythmic stimuli. While the authors suggested that their results might support an underwater sound localization hypothesis, measurements comparable to those of the pinnipeds were also obtained for a domestic dog (*Canis familiaris*). They concluded, therefore, that it is possible that temporal resolution in pinnipeds may not be the result of the evolutionary pressure of an aquatic environment but, rather, a result of increased high-frequency hearing essential to carnivore-sound localization.

Cetaceans are not the only marine mammals that can benefit from AEP analysis. This technique recently has been shown to be also appropriate for use in pinnipeds. **Reichmuth et al.** examined some of the basic measurement and response characteristics of the ABR in pinnipeds. The subjects were California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*), and northern elephant seals (*Mirounga angustirostris*) that were awake, sedated, or anesthetized during in-air testing. Results indicated that the ABRs were of highest amplitude when measured from subdermal electrodes. The ABR waveforms were generally similar among the species tested, although the amplitude of the elephant seal ABR was much smaller than that of the other two species at similar stimulus levels. Bandpass filtering of the ABR resulted in improved signal-to-noise ratios but also caused reduction in response amplitude and distortion of the ABR waveform at high-pass settings of 100 Hz. Five-cycle tone bursts provided the best tradeoff between stimulus bandwidth and frequency specificity. The amplitude of ABRs evoked by clicks and tone bursts as a function of stimulus level was approximately linear for California sea lions and harbor seals over a range of ~25 dB re 1 µPa.

All these manuscripts contribute to show the potential of AEP techniques to assess hearing in marine mammals. Electrophysiological methods can be adapted and applied for almost any marine mammal species. They offer an attractive, though non-straightforward for the non-expert, alternative to behavioural and psychophysical measurements. Much research still needs to be done to confidently measure the acoustic sensitivity of pinnipeds, sirenians, sea otters, manatees, polar bears, and many cetacean species—in particular, to understand auditory processes in baleen whales. While noise pollution sources from human activities overlap frequencies that are used by mysticetes to communicate and perhaps orient themselves, basic data are lacking on how they receive and process sounds as well as the range of their frequency and temporal resolution capabilities. The development of portable autonomous AEP units may help in accessing these fundamental data in the future.

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