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RESEARCH ARTICLE

Influence of fatiguing noise on auditory evoked responses to stimuli of various levels in a beluga whale, *Delphinapterus leucas*

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ABSTRACT

The negative impact of man-made noise on the hearing of odontocetes has attracted considerable recent attention. In the majority of studies, permanent or temporary reductions in sensitivity, known as permanent or temporary threshold shift (PTS or TTS, respectively), have been investigated. In the present study, the effects of a fatiguing sound on the hearing of a beluga whale, *Delphinapterus leucas*, within a wide range of levels of test signals was investigated. The fatiguing noise was half-octave band-limited noise centered at 32 kHz. Post-exposure effects of this noise on the evoked responses to test stimuli (rhythmic pip trains with a 45-kHz center frequency) at various levels (from threshold to 60 dB above threshold) were measured. For baseline (pre-exposure) responses, the magnitude-versus-level function featured a segment of steep magnitude dependence on level (up to 30 dB above threshold) that was followed by a plateau segment that featured little dependence on level (30 to 55 dB above threshold). Post-exposure, the function shifted upward along the level scale. The shift was 23 dB at the threshold and up to 33 dB at the supra-threshold level. Owing to the plateau in the magnitude-versus-level function, post-exposure suppression of responses depended on the stimulus level such that higher levels corresponded to less suppression. The experimental data may be modeled based on the compressive non-linearity of the cochlea. According to the model, post-exposure responses of the cochlea to high-level stimuli are minimally suppressed compared with the pre-exposure responses, despite a substantially increased threshold.

KEY WORDS: TTS, Auditory evoked responses, Dolphin, Hearing

INTRODUCTION

The negative impact of man-made noise on the behavior and physiology of aquatic animals is manifested in a variety of effects. Considerable recent attention has been paid to the impact of man-made noise on the hearing of cetaceans, particularly odontocetes. The sensitive auditory system of odontocetes, which is used for both passive hearing and echolocation (Au, 1993; Nachtigall et al., 2000; Supin et al., 2001; Au and Hastings, 2008), may be particularly susceptible to damage caused by intensive noise. The effects of noise on the auditory system resulting in permanent or temporary reductions in sensitivity are known as permanent or temporary threshold shifts (PTS or TTS, respectively). In terms of wildlife protection and conservation, PTS is the most important phenomenon because it reduces capabilities of the leading

sensory system used for orientation, communication and foraging. However, PTS has not been specifically studied in marine mammals for obvious ethical reasons. Instead, the reversible effect (TTS) in odontocetes has been a matter of active investigation under the assumption that a better understanding of the conditions leading to TTS might help to predict the conditions for PTS.

To date, many TTS studies have been performed on several species of odontocetes. These studies are summarized in a recent review by Finneran (2015). Previous studies have revealed many features of TTS and its dependence on the fatiguing noise level, duration, frequency contents and manner of presentation (continuous or intermittent). This knowledge has allowed an elaboration of several criteria for assessing the impact of noise on the hearing of odontocetes (reviews by Southall et al., 2007; Finneran and Jenkins, 2012; Finneran, 2015).

The majority of the studies have focused on the impact of fatiguing sounds on hearing thresholds. Such studies have been performed using two methods of measurement: psychophysical measurements and auditory evoked potentials (AEP). In psychophysical TTS studies, threshold measurements have been primarily exploited. Although there have been attempts to measure supra-threshold 'subjective loudness' in a bottlenose dolphin (Finneran and Schlundt, 2011), the influence of fatiguing sounds on the hearing of supra-threshold signals has never been investigated psychophysically. Many AEP investigations of TTS have included recording the responses to several supra-threshold stimuli, but when the impact of fatiguing noise was assessed, it was performed in terms of threshold evaluation. In a study by Finneran et al. (2007), noise effects on the auditory steady-state evoked response were investigated within a range of levels from threshold to more than 120 dB re. 1 µPa. However, the vast majority of TTS investigations that have utilized the AEP method have focused on thresholds.

In the wild, animals rely on supra-threshold signals for orientation, foraging and communication. Data on the impact of noise on hearing thresholds are important for predicting supra-threshold perception. However, these predictions are not comprehensive because of the complex and non-linear dependence of hearing on signal parameters. Therefore, the influence of noise on the perception of supra-threshold signals in odontocetes requires special investigation.

The goal of the present study was to investigate the effects of a fatiguing sound on the hearing of an odontocete subject within a wide range of levels of test signals. To accomplish this goal, we recorded and measured AEPs to sound stimuli of various levels in a beluga whale [*Delphinapterus leucas* (Pallas 1776)] before and after exposure to fatiguing noise.

MATERIALS AND METHODS
Subject and facilities
The subject was a 3.5-year-old female beluga whale, *D. leucas*, with a body length of 270 cm and body mass of 340 kg, kept at the Utrish

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Marine Station of the Russian Academy of Sciences on the Black Sea coast. The animal was housed in a round seawater tank 6 m in diameter and 1.5 m in depth. The care and use of the animal were in compliance with the Guidelines of the Russian Ministry of Education and Science on the use of animals in biomedical research.

Test stimuli
The test stimuli were tone pip trains digitally generated at an update rate of 512 kHz. The trains were 16 ms long and contained 16 pips presented at a rate of 1000 pips s^{-1} (Fig. 1A). Each pip in the train included eight cycles of a 45 kHz carrier enveloped by a cosine function (Fig. 1B). The overall duration of each pip was 0.18 ms, and its equivalent rectangular duration was 0.09 ms. The frequency band of the main spectrum lobe of the generated pips (Fig. 1C) was
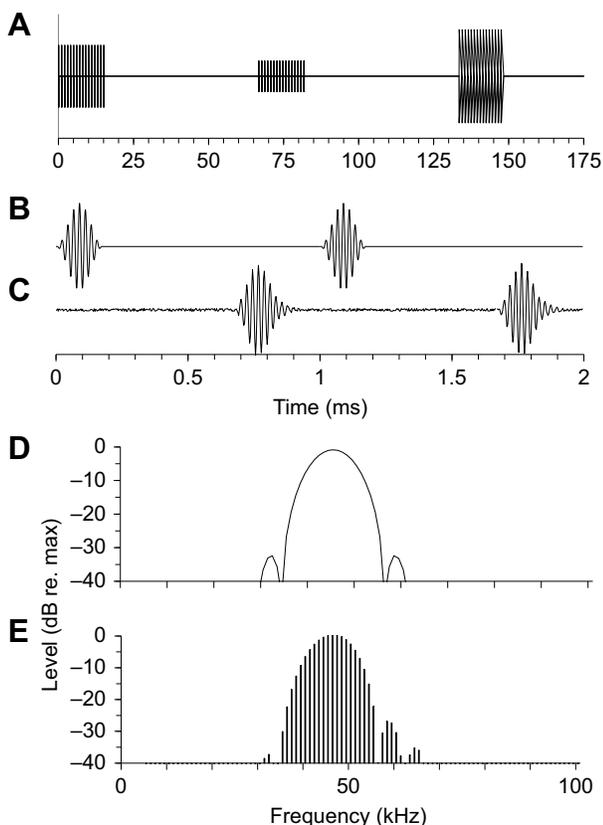
Fig. 1. Stimulus waveform and spectrum. (A) Succession of stimulus pip trains; an arbitrary fragment containing three trains of long train succession is exemplified. Note train-by-train variation of stimulus level. (B) Pip waveform (electronic signal); a fragment containing two pips of a 16-pip train is exemplified. (C) Pip waveform (acoustic signal). Ordinates in B and C are arbitrary. (D) Frequency spectrum of a single pip (electronic signal). (E) Frequency spectrum of a pip train (acoustic signal).

35 to 54 kHz at a level of −20 dB. The spectrum of the acoustic stimulus (Fig. 1D) did not precisely reproduce that of the electronic signal (Fig. 1C) because of the frequency response of the transducer (see 'Instrumentation', below), and therefore, its frequency band was 36 to 55 kHz at a level of −20 dB. The test stimuli as trains of short pips were used because they produced a rhythmic AEP sequence referred to as the rate-following response (RFR) more effectively than narrow-band sinusoidally modulated tones (Supin and Popov, 2007).

The pip level was constant throughout each train; however, throughout a trial, the level was randomly varied train-by-train within a range of 35 dB in eight 5 dB steps. Depending on the amplification and/or attenuation of the play-back channel, this range of variation resulted in across-train root mean square (RMS) sound pressure levels (SPL) of either 65–100 dB or 90–125 dB (here and below, all SPLs are specified in dB re. 1 μPa).

The pip trains were presented at a rate of 15 trains s^{-1}. In a separate channel, a pulse for triggering coherent AEP acquisition was generated, and the trigger pulses coincided with the onset of each pip train.

Fatiguing noise
The fatiguing noise was digitally generated at a sampling rate of 512 kHz. It was half-octave band-filtered noise (a fourth-order Butterworth filter) with a frequency band of 27 to 38 kHz, and thus its logarithmic center frequency was 32 kHz, which was a half-octave below the center frequency of the test signals. This frequency band for the fatiguing noise was chosen because a previous investigation revealed high effectiveness of a noise half-octave below the test signal in producing TTS in belugas (Popov et al., 2013). The noise RMS SPL was 170 dB, the exposure duration was 10 min. Thus, the sound exposure level (SEL) was 198 dB re. 1 μPa²s.

Acoustic measurements
The SPLs of the pip trains were specified in dB RMS re. 1 μPa over the 16-ms pip-train duration. Computation of the RMS across the entire train duration was used because at the pip rate of 1000 s^{-1} RFR features nearly complete energy summation over both the pips and pauses of the train. Therefore, threshold estimates specified in RMS over the entire train duration agree with those provided by other methods (Supin and Popov, 2007). The SPL of the test stimuli was monitored before and after several experiments by positioning a receiving hydrophone (see Instrumentation, below) next to the animal's head. Despite the sound reflections within the tank, local sound levels around the animal's head varied within a range of ±2.5 dB.

The SPL of the fatiguing noise was also specified in dB re. 1 μPa RMS and was monitored in the same manner as the test stimuli.

Evoked potential recording
Brain potentials were picked up through surface disk electrodes (see Instrumentation, below). The active electrode was positioned at the vertex, 7 cm behind the blowhole and above the water surface. The reference electrode was positioned on the back. Brain potentials were fed through shielded cables to a balanced amplifier with an 80 dB gain and a frequency passband of 100 to 3000 Hz, digitized and processed with a standard personal computer.

For extraction of responses from the brain potentials, the processing routine extracted 25 ms epochs in synchrony with the onset of the test pip train. The epochs were sorted online in eight

bins according to the level of the stimulus. During each trial, a total of 4000 epochs were collected, i.e. 500 epochs in a bin for each of the eight stimulus levels. In each bin, the epochs were averaged online, which resulted in eight records being collected simultaneously in an interleaving manner. At the stimulus presentation rate of 15 s^{-1}, the collection of the total of 4000 epochs took 267 s; thus, collections could be repeated every 4.5–5 min. A 16 ms segment of the averaged records (from the sixth to the 22nd millisecond after triggering) containing the RFR to the pip train stimuli were fast Fourier transformed online to obtain the response frequency spectrum. With the 16 ms analyzed window and 16 kHz sampling rate (256 samples in the window), the spectrum resolution was 62.5 Hz. The 1 kHz spectral peak was considered as a measure of the RFR magnitude.

Experimental design

During experimentation, the subject remained in the housing tank. The water level in the tank was lowered to 50 cm. The animal was supported by a stretcher so that the dorsal part of the body and the blowhole were above the water surface. The stretcher was transparent to sound (made of a fishing net). The animal was not anaesthetized.

The transducer that served to play both the test signals and the fatiguing noise was immersed in water at a depth of 25 cm, 1 m in front of the animal's head.

Each experiment began by recording the pre-exposure responses within a stimulus level range from 65 to 125 dB SPL. These records were collected in two trials: one with a random variation of the stimulus level from 65 to 100 dB SPL and the other with a random variation of the stimulus level from 90 to 125 dB SPL. Then, the animal was exposed to the fatiguing noise for 10 min. After exposure, recording trials followed immediately one after another. Responses to test stimuli were collected every 4.5–5 min. During recording, the frequency spectra of the records were obtained online, thus allowing online monitoring of the response collection. Depending on the result obtained in each trial, either the same range of stimulus level was used in successive trials (in this case, thresholds were determined every 4.5–5 min), or both stimulus level ranges (65–100 dB and 90–125 dB) were used in successive trials (in the latter case, thresholds were determined every 9–10 min).

Post-exposure investigation lasted no longer than 1 h, even if total recovery was not achieved. This time limit was applied to restrict the time during which the animal was kept immobile on a stretcher. The session including pre-exposure investigation, exposure, post-exposure investigation and accompanying manipulation lasted no longer than 2 h. Within this time limit, the animal lay quietly on the stretcher throughout the session, and no disturbance in behavior was observed upon its release and refilling of the home tank. During each experimental session, the fatiguing noise was presented once. Eight identical sessions were carried out, one session a day, 1 or 2 days apart. With these inter-session intervals, baseline thresholds and supra-threshold responses completely recovered from session to session (see Results).

Instrumentation

Both test and fatiguing sounds were digitally synthesized by a standard personal computer using a custom-made program (Virtual Instrument) designed using LabVIEW software (National Instruments, Austin, TX, USA). The synthesized signals were digital-to-analog converted by a D/A channel of an NI USB-6251 acquisition board (National Instruments). To amplify and attenuate the test signal, a custom-made low-noise amplifier-attenuator with a

200 kHz passband and 50 Ω output impedance was used. To amplify the fatiguing sound, a CV-1800 amplifier (Cerwin-Vega, USA) with a 60 kHz passband was used. Both test signals and fatiguing noise were played through a B&K 8104 transducer (Bruel & Kjaer, Naerum, Denmark). The playback channel was calibrated by a receiving hydrophone (B&K 8103, Bruel & Kjaer) with a custom-made amplifier with a 40 dB gain and a 200 kHz passband based on an AD820 chip (Analog Devices, Norwood, TX, USA).

Brain potentials were obtained using F-E5G 10-mm golden plated disk electrodes (Grass Technologies, Warwick, RI, USA) and an LP511 brain potential amplifier (Grass Technologies). Digitizing of the amplified signals was performed with a 16 bit analog-to-digital converter, which was one of the A/D channels of the same NI USB-6251 acquisition board employed for sound generation. Processing of the digitized signals was performed using a custom-made program (Virtual Instrument) designed using LabVIEW software (National Instruments).

RESULTS

Pre- and post-exposure responses to various stimulus levels

In baseline (pre-exposure) trials, the pip-train stimuli evoked RFR as a rhythmic sequence of AEP waves of the same frequency as the pip rate (1000 Hz), as exemplified in Fig. 2A. At low levels (approximately 75–100 dB SPL in Fig. 2A), the response magnitude grew as the stimulus level increased. With further increases in the stimulus levels (100–120 dB re. 1 µPa in Fig. 2A), the response magnitude growth was slow or negligible. Within the range of levels of 120–125 dB re. 1 µPa, the response again grew.

Shortly after exposure (5 min), a substantial reduction in the responses compared with the pre-exposure responses occurred. The reduction was the most obvious at lower stimulus levels. In the experiment exemplified in Fig. 2, RFR was not detectable at
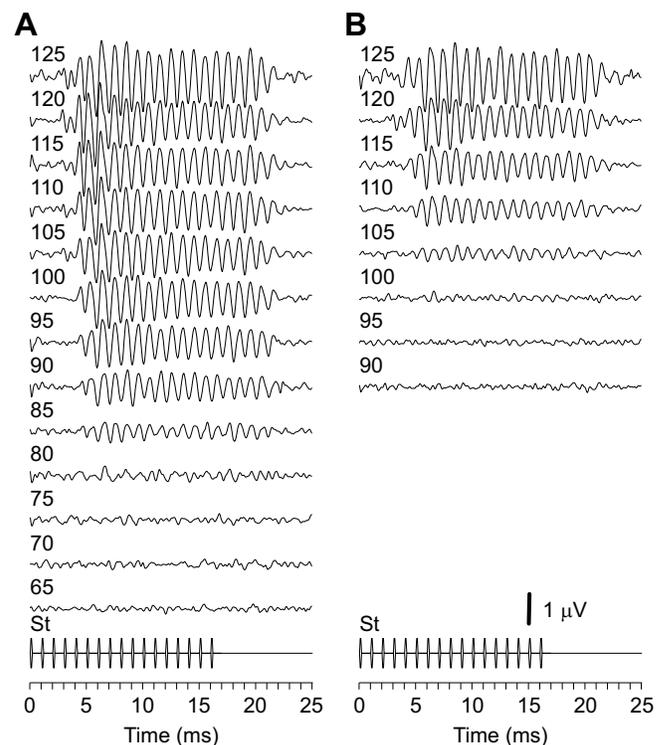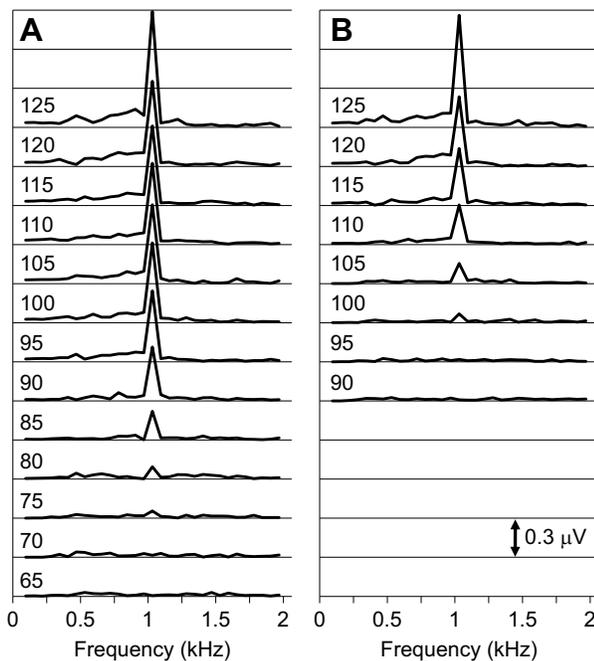
Fig. 2. Rate-following response waveforms. (A) Baseline session. (B) 5 min post-exposure. Stimulus levels (dB SPL) are indicated next to the records. St, stimulus envelope.

stimulus levels of 95 dB SPL and lower (Fig. 2B), whereas before exposure, stimuli at those levels evoked robust responses (Fig. 2A). Thus, the response threshold increased by approximately 25 dB, demonstrating a typical TTS effect. At stimulus levels of 100–110 dB re. 1 µPa, the post-exposure responses were several times lower than the pre-exposure responses to the same stimulus levels. At high stimulus levels, the difference between pre- and post-exposure responses was less obvious.

Dependence of pre- and post-exposure responses on stimulus level

The pre- and post-exposure responses were quantified by computing their frequency spectra, as exemplified in Fig. 3, which presents the spectra of waveforms from Fig. 2. At supra-threshold stimulus levels, the spectra featured a definite peak at a frequency of 1 kHz that revealed the RFR to 1000 s^{-1} pip trains. The spectra of the pre-exposure responses confirmed the appearance of a response at the stimulus level of 75 dB SPL, steep response growth with increasing stimulus levels up to 100 dB SPL, slow response growth (nearly a plateau) with increasing stimulus levels up to 120 dB SPL, and then growth again at a stimulus level of 125 dB SPL. A post-exposure response appeared at stimulus levels 100 dB SPL and higher, thus revealing a TTS of approximately 25 dB. Responses progressively grew with increasing stimulus levels up to the maximal tested level of 125 dB SPL.

The pre- and post-exposure RFR magnitudes evaluated based on the RFR frequency spectra are presented as a function of the stimulus level in Fig. 4A. The baseline (pre-exposure) and 5 min post-exposure plots present the same data as shown in Figs 2 and 3. Additionally, magnitude-versus-level functions are presented for post-exposure times of 15, 25 and 50 min. After exposure and during subsequent recovery, modification of the magnitude-versus-level function primarily appeared as a shift along the level axis, first to higher levels (5 min post-exposure) and then back to lower levels during recovery (15–50 min). As a result of this shift, shortly after
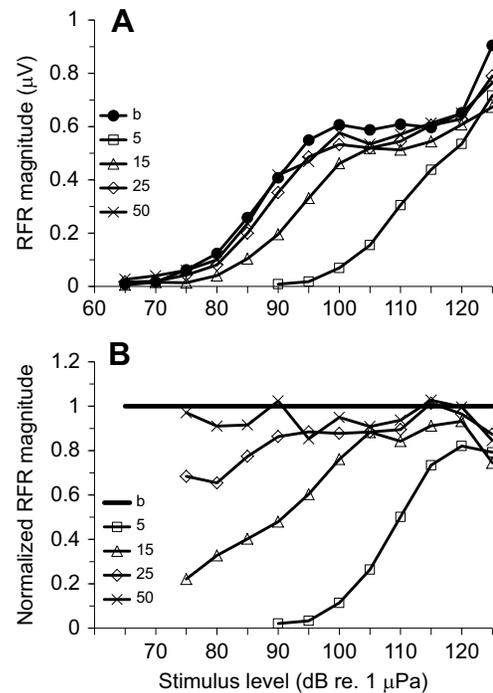
Fig. 4. Magnitude-versus-level functions for the records exemplified in Figs 2 and 3. (A) Response magnitudes presented in voltage. (B) The same data presented as a ratio of post-exposure to baseline response magnitude (baseline response magnitudes are taken as 1 by definition). b, baseline function; 5, 15, 25 and 50, functions for 5 to 50 min post-exposure. RFR, rate-following response.
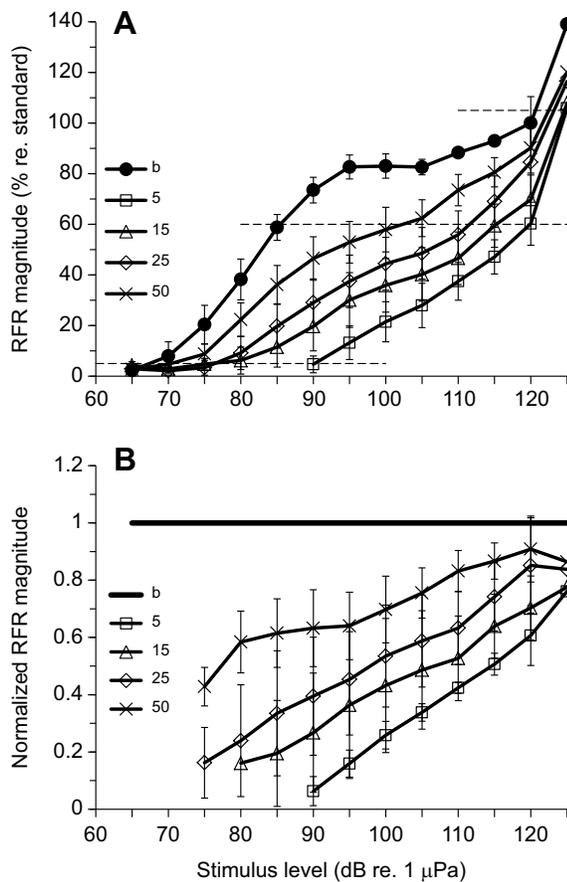
exposure (5 min), responses to low-level stimuli (up to 90 dB re. 1 µPa) were completely suppressed. Responses gradually recovered at longer post-exposure times. Alternatively, for high-level stimuli (115–125 dB re. 1 µPa), the post-exposure responses at all post-exposure times differed minimally from the pre-exposure responses.

Dependence of the exposure effect on the stimulus level was obvious when the magnitudes of the post-exposure responses were normalized relative to the pre-exposure responses to the same stimulus level (Fig. 4B). Both shortly after exposure (5 min) and during recovery (15 to 50 min), high stimulus levels resulted in less post-exposure response reduction.

The results of all eight experimental sessions are summarized in Fig. 5. To compensate for session-by-session fluctuations in response magnitude, all magnitudes were presented as the percentage of the baseline response in the same session to a stimulus level of 120 dB; these normalized magnitudes were averaged across sessions.

The averaged baseline magnitude-versus-level function (Fig. 5A) featured small standard errors, thus indicating session-by-session recovery of the original functions. The averaged function demonstrated a mean threshold of 65 dB, then an oblique segment at lower (65–95 dB) stimulus SPLs which are 0–30 dB above the 65 dB threshold, a shallow-slope (plateau) segment at higher (95–120 dB SPL) levels (30–55 dB above threshold), and one more oblique segment from 120 to 125 dB SPL (55–60 dB above threshold).

Post-exposure functions were shifted relative to the baseline function. For high response magnitudes, the shift was slightly larger than for low response magnitudes, i.e. the steepness of the shifted functions was slightly less than for the baseline. Specifically,

Fig. 3. Frequency spectra of the records exemplified in Fig. 2A and B, respectively. (A) Baseline session. (B) 5 min post-exposure. Stimulus levels (dB SPL) are indicated next to the spectra.

Fig. 5. Magnitude-versus-level functions, means of eight sessions.
(A) Response magnitudes presented as percentage of a standard; the standard was the baseline response magnitude at a stimulus level of 120 dB SPL. Horizontal dashed lines depict shifts of the magnitude-versus-level functions for magnitudes of 5, 60 and 105% of the standard. (B) The same data presented as a ratio of post-exposure to baseline response magnitude (baseline response magnitudes are taken as 1 by definition). Error bars are ±s.e.m. b, baseline function; 5, 15, 25 and 50, functions for 5 to 50 min post-exposure.

shortly after exposure (5 min), the near-threshold (response magnitude of 5%) shift was approximately 25 dB (from 65 to 90 dB SPL); for a 60% response magnitude, the shift was as large as 35 dB (from 85 to 120 dB SPL). For high-magnitude responses (105%) evoked by high-level (120–125 dB SPL) stimuli, the post-exposure shift was minor, approximately 5 dB. Later after exposure (15–50 min), the functions gradually shifted back to the baseline function.

The dependence of the exposure effect on stimulus level is demonstrated in Fig. 5B, where averaged post-exposure response magnitudes are presented as a proportion of the baseline responses to the same stimulus levels. Similar to Fig. 4, the plots directly demonstrate that higher stimulus levels resulted in less the post-exposure response reduction.

Pre- and post-exposure thresholds and iso-magnitude levels
Based on the obtained data, pre- and post-exposure iso-magnitude levels were evaluated. The averaged baseline response magnitude at a stimulus level of 120 dB SPL was taken as a 100% standard, and stimulus levels that evoked response of 5, 20, 40, 60, 80 and 100% of the standard were found as functions of the post-exposure time. A magnitude of 5% of the standard was taken as a threshold

response. These threshold estimates differed from our previous studies, which used regression lines for magnitude-versus-level functions (Supin et al., 2001; Supin and Popov, 2007). This method was used herein to make comparable estimates for both threshold and supra-threshold iso-magnitude lines and assuming that for TTS studies, irrespective of the threshold estimate method, threshold shifts are more important than absolute threshold values (which are always arbitrary to some extent). The iso-magnitude stimulus levels were calculated by interpolation between the levels above and below the specified response magnitude.

The results of this procedure are presented in Fig. 6. Short after exposure (5 min), the threshold estimate increased by 23 dB compared with the pre-exposure threshold (91 versus 68 dB SPL), thus demonstrating a typical TTS effect. Then, the thresholds recovered gradually. For higher response magnitudes (20–60%), the stimulus levels increased even more than for the thresholds: for the 60% response magnitude, the increase was 33 dB (from 87 to 120 dB SPL). However, for the highest response magnitude (100%), the stimulus levels were minimally affected by noise exposure: from 120.0 to 128 dB SPL, i.e. by 8 dB.

DISCUSSION
Restriction for the investigated level range
A feature of the present data was a higher baseline threshold (65 dB re. 1 µPa) than the thresholds of 45 to 50 dB found in our previous investigations in belugas at the same frequency and with the use of the same threshold-determination technique (Popov et al., 2013, 2016). Most probably, the increased baseline threshold in the present study is a result of the method of the test stimulus presentation: during each trial, stimuli of randomly varied levels were presented in an interleaving manner (see Materials and methods, Test stimuli). This stimuli presentation could result in hearing adaptation to supra-threshold sound levels (Popov et al., 2016) manifesting in the increased threshold estimate that characterized the hearing sensitivity not in silence but in a certain sound background. Despite this disadvantage, we used the interleaving manner of stimulus presentation because, otherwise, post-exposure recovery of responses to different stimulus level could not be traced equally.

With the across-session averaged baseline threshold of 65 dB re. 1 µPa, we restricted variations in the stimulus level to a
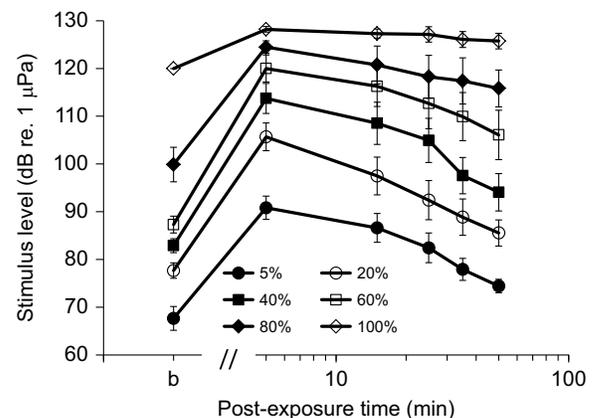
Fig. 6. Iso-magnitude lines: stimulus levels producing responses of a specified magnitude as functions of post-exposure time (b, baseline).
Response magnitudes (5 to 100%, as indicated in the level) are presented as percentage of a standard; the standard was the baseline response magnitude at a stimulus level of 120 dB SPL.

limit of 125 dB SPL, which corresponded to approximately 60 dB above the threshold of 65 dB re. 1 µPa. A reason for this restriction was the typical amplitude-versus-level function for the RFR. This function includes a near-threshold segment of response dependence on stimulus level, then a segment of little or no dependence (plateau), and finally, at high stimulus levels, one more segment of steep response growth with level (Supin et al., 2001; Supin and Popov, 2007; Finneran et al., 2007) (see Fig. 5A). The origin of the segment at high stimulus levels is questionable. Finneran et al. (2007) considered the amplitude-versus-level function as a sum of two processes in the auditory system: a saturating process responsible for the near-threshold and plateau segments at lower stimulus levels, and a linear process responsible for the steep-dependence segment at high stimulus levels. According to Supin et al. (2001), the segment of steep dependence may appear because of stimulation of the cochlear partition far beyond the nominal signal frequency band. In the present study, the steep-dependence segment appeared at stimulus levels from 120 to 125 dB re. 1 µPa. Because the nature of the steep-dependence segment is not known, interpretation of the data obtained at such stimulus levels is dubious. Therefore, in the present study, we did not use stimulus levels above 125 dB SPL and considered only data obtained at stimulus levels up to 120 dB SPL as trustworthy. The data obtained at 125 dB SPL were omitted from further consideration.

Noise impact on responses to different stimulus levels

Within the stimulus level range of 65 to 120 dB (0 to 55 dB above baseline threshold), the effect of noise exposure appeared as a shift in the magnitude-versus-level function toward higher stimulus levels. The shift was from 25 dB for a near-threshold response magnitude to 35 dB at a higher (60% of the maximum) response magnitude. This effect was similar to that described by Finneran et al. (2007). Owing to the presence of a response-magnitude plateau at stimulus levels from 95 to 120 dB, the shift of the magnitude-versus-level function resulted in a deep suppression of responses to lower-level (65–90 dB SPL) stimuli and minor suppression of responses to higher-level (110–120 dB SPL) stimuli. Therefore, we questioned whether these data indicate that post-exposure responses of the cochlea to high-level stimuli are nearly the same (or slightly reduced) as the pre-exposure response, despite a substantial increase in the threshold.

The answer to this question depends on the nature of the plateau in the response magnitude-versus-level functions. This plateau has been shown to be typical of various versions of AEP in odontocetes. The auditory brainstem response (ABR) to band-limited sound pulses features obvious dependence on the stimulus level within a range of 20–25 dB followed by a plateau in the magnitude-versus-level function (Popov and Supin, 2001). RFR, which is a rhythmic train of ABRs, is dependent on the stimulus level only within a short near-threshold range (Supin and Popov, 1995). Finneran et al. (2007) interpreted their findings in the same way. The nature of this form of response magnitude-versus-level function may be searched for in basic hearing mechanisms.

As a hypothesis, we suggest that this search may be associated with a basic cochlear mechanism known as non-linear compression. Non-linear compression is a feature of the active cochlear mechanism that is based on the activity of outer hair cells. The gain of the active mechanism depends on the stimulus level, where higher levels correspond to lower gain (Robles and Ruggero 2001; Cooper, 2004; Moore, 2013).

The dependence of the active gain mechanism on the stimulus level has never been investigated in odontocetes. Therefore, as a first

approximation, we assumed this dependence to be the same or similar to that in humans. A family of gain-versus-level functions for human hearing was presented in a study by Glasberg and Moore (2000). Those functions were presented for different gain limits, G_0, from 20 to 60 dB. In consideration of the high sensitivity of odontocete hearing, we assumed $G_0=60$ dB (Fig. 7). The assumption of $G_0=60$ dB means that the threshold stimulus produces vibration of the basilar membrane 60 dB as high as that produced by the same stimulus without the active mechanism ($G_0=0$ dB).

Fig. 7 demonstrates that at $G_0=60$ dB, the magnitude-versus-level function qualitatively resembles the baseline experimental function obtained in the present study. It predicts steep growth of the response at stimulus levels from 0 to approximately 20 dB above threshold and much less growth (quasi plateau) at higher (up to approximately 70 dB) levels. As a result, a response to a 25 dB stimulus (magnitude 1) is as high as 85% of a response to a 50 dB stimulus (magnitude 2). The function also predicts increased response growth at stimulus levels above 80 dB.

It is not known yet which mechanisms are involved in TTS in odontocetes for the parameters of fatiguing sound used in the present study. As a hypothesis, we propose that the fatiguing exposure may result in decreased gain of the active cochlear mechanism. This suggestion does not contradict cochlear physiology because the active mechanism is expected to be more sensitive to fatiguing effects than the passive mechanism based on hydro-mechanical processes in the cochlea. Suppose that after exposure, G_0 of the active mechanism fell from 60 dB to 40 dB; then, the gain-versus-level function would look as shown in Fig. 7 with $G_0=40$ dB. This function reaches the threshold level at a stimulus level of 25 dB, i.e. it models the 25 dB TTS. At a 50 dB stimulus level (25 dB above the shifted threshold), the function models a slightly decreased response magnitude: 88% of the baseline response to the 25 dB stimulus and 74% of the baseline response to the 50 dB stimulus. Considering the scatter of the
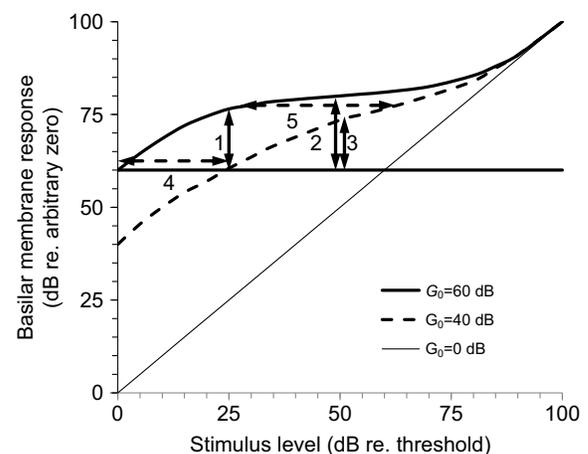
Fig. 7. Modeling of dependence of pre- and post-exposure cochlear response on stimulus level. Dependence of basilar membrane response on stimulus level are presented according to a model by Glasberg and Moore (2000) for three gain limits, G_0, of 60, 40 and 0 dB, supposing $G_0=60$ dB before and $G_0=40$ dB after exposure. Vertical double-headed arrows: 1, pre-exposure basilar membrane response magnitude to a stimulus of 25 dB above threshold; 2, pre-exposure response to a stimulus of 50 dB above threshold; and 3, post-exposure response to a stimulus of 50 dB above pre-exposure threshold (25 dB above post-exposure threshold). Horizontal double-headed arrows: 4, function shift at the threshold level; and 5, function shift at a level 17.5 dB above threshold.

experimental data (see error bars in Fig. 5), these results satisfactorily mimic the experimental data. Note also that in Fig. 7, the shift for supra-threshold responses is greater than that at the threshold (35 dB above threshold, contrary to 25 dB at the threshold). This also fits the experimental data.

Thus, the model based on the non-linear cochlear compression satisfactorily simulates both the pre- and post-exposure magnitude-versus-level functions. It explains the small post-exposure suppression of the responses of the cochlea to high-level stimuli despite the substantial threshold shift.

Implications for noise impact on stimulus discrimination

As follows from the experimental data, fatiguing noise exposure not only increases hearing thresholds but also reduces responses to supra-threshold signals. It is remarkable that, according to the data presented above, the moderate-level stimuli are markedly affected by fatiguing sounds. The signals of moderate levels, i.e. the levels between threshold and the upper perceptible limit, are the signals to which the auditory system is evolutionary adapted; they may carry the most obvious information on signal properties, which are important cues for signal discrimination. Therefore, it may be suggested that the impacts of fatiguing sounds on the hearing of odontocetes are not limited by reduced capability to detect faint sounds (increased thresholds) and include deterioration of signal discrimination.

References

Au, W. W. L. (1993). *The Sonar of Dolphins*. New York: Springer-Verlag.

Au, W. W. L. and Hastings, M. C. (2008). *Principles of Marine Bioacoustics*, 677 pp. New York: Springer-Science.

Cooper, N. P. (2004). Compression in the peripheral auditory system. In *Compression: From Cochlea to Cochlear Implants* (ed. S. P. Bacon, R. R. Fay and A. N. Popper), pp. 18-61. New York: Springer-Verlag.

Finneran, J. J. (2015). Noise-induced hearing loss in marine mammals: a review of temporary threshold shift studies from 1996 to 2015. *J. Acoust. Soc. Am.* **138**, 1702-1726.

Finneran, J. J. and Jenkins, A. K. (2012). *Criteria and Thresholds for U.S. Navy Acoustic and Explosive Effects Analysis*. San Diego: Space and Naval Warfare Systems Center.

Finneran, J. J. and Schlundt, C. E. (2011). Subjective loudness level measurements and equal loudness contours in a bottlenose dolphin (*Tursiops truncatus*). *J. Acoust. Soc. Am.* **130**, 3124-3136.

Finneran, J. J., Schlundt, C. E., Branstetter, B. and Dear, R. L. (2007). Assessing temporary threshold shift in a bottlenose dolphin (*Tursiops truncatus*) using multiple simultaneous auditory evoked potentials. *J. Acoust. Soc. Am.* **122**, 1249-1264.

Glasberg, B. R. and Moore, B. C. J. (2000). Frequency selectivity as a function of level and frequency measured with uniformly exciting notched noise. *J. Acoust. Soc. Am.* **108**, 2318-2328.

Moore, B. C. J. (2013). *An Introduction to the Psychology of Hearing*. Brill: Leiden Boston.

Nachtigall, P. E., Lemonds, D. W. and Roitblat, H. L. (2000). Psychoacoustic studies of dolphin and whale hearing. In *Hearing by Whales and Dolphins* (ed. W. W. L. Au, A. N. Popper and R. R. Fay), pp. 330-363. New York: Springer-Verlag.

Popov, V. V. and Supin, A. Y. (2001). Contribution of various frequency bands to ABR in dolphins. *Hear. Res.* **151**, 250-260.

Popov, V. V., Supin, A. Y., Rozhnov, V. V., Nechaev, D. I., Sysuyeva, E. V., Klishin, V. O., Pletenko, M. G. and Tarakanov, M. B. (2013). Hearing threshold shifts and recovery after noise exposure in beluga whales, *Delphinapterus leucas*. *J. Exp. Biol.* **216**, 1587-1596.

Popov, V. V., Sysueva, E. V., Nechaev, D. I., Rozhnov, V. V. and Supin, A. Y. (2016). Auditory evoked potentials in the auditory system of a beluga whale *Delphinapterus leucas* to prolonged sound stimuli. *J. Acoust. Soc. Am.* **139**, 1101-1109.

Robles, L. and Ruggero, M. A. (2001). Mechanics of the mammalian cochlea. *Physiol. Rev.* **81**, 1305-1352.

Southall, B. L., Bowels, A. E., Ellison, W. T., Finneran, J. J., Gentry, R. I., Greene, C. R., Jr, Kastak, D., Ketten, D. R., Miller, J. H., Nachtigall, P. E. et al. (2007). Marine mammal noise exposure criteria: Initial Scientific Recommendations. *Aquat. Mamm.* **33**, 411-521.

Supin, A. Y. and Popov, V. V. (1995). Envelope-following response and modulation transfer function in the dolphin's auditory system. *Hear. Res.* **92**, 38-46.

Supin, A. Y. and Popov, V. V. (2007). Improved techniques of evoked-potential audiometry in odontocetes. *Aquat. Mamm.* **33**, 17-26.

Supin, A. Y., Popov, V. V. and Mass, A. M. (2001). *The Sensory Physiology of Aquatic Mammals*. Boston: Kluwer.