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RESEARCH ARTICLE

Hearing threshold shifts and recovery after noise exposure in beluga whales, *Delphinapterus leucas*

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SUMMARY

Temporary threshold shift (TTS) after loud noise exposure was investigated in a male and a female beluga whale (*Delphinapterus leucas*). The thresholds were evaluated using the evoked-potential technique, which allowed for threshold tracing with a resolution of ~1 min. The fatiguing noise had a 0.5 octave bandwidth, with center frequencies ranging from 11.2 to 90 kHz, a level of 165 dB re. 1 μPa and exposure durations from 1 to 30 min. The effects of the noise were tested at probe frequencies ranging from –0.5 to +1.5 octaves relative to the noise center frequency. The effect was estimated in terms of both immediate (1.5 min) post-exposure TTS and recovery duration. The highest TTS with the longest recovery duration was produced by noises of lower frequencies (11.2 and 22.5 kHz) and appeared at a test frequency of +0.5 octave. At higher noise frequencies (45 and 90 kHz), the TTS decreased. The TTS effect gradually increased with prolonged exposures ranging from 1 to 30 min. There was a considerable TTS difference between the two subjects.

Key words: whale, hearing, noise, threshold shift.

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INTRODUCTION

The negative impact of man-made noises on the behavior and physiology of aquatic animals manifests itself in a variety of ways. The impact of man-made noise on the hearing of cetaceans, particularly odontocetes, deserves special attention. The sensitive and wide-ranging frequency auditory system of odontocetes, which is used for both passive hearing and echolocation, might be particularly susceptible to damage by intensive noise. The impact of noise on this auditory system, resulting in the permanent or temporary reduction of sensitivity, is known as a permanent (PTS) or temporary threshold shift (TTS). In terms of wildlife protection and conservation, PTS is the most important phenomenon. However, PTS has not been intentionally studied in marine mammals. Instead, reversible effects (i.e. TTS) in odontocetes have been a subject of active investigation under the assumption that a better understanding of conditions for TTS might help to predict conditions for PTS.

Many factors influence the TTS effects, including noise center frequency and bandwidth, noise level, noise exposure duration, time after noise exposure, continuous or intermittent exposure, test frequency of TTS measurement and subject species. The dependence of TTS on these factors has been extensively investigated in humans and laboratory mammals (reviewed by Miller et al., 1963; Clark, 1991; Melnick, 1991; Yost, 1994). However, applicability of the found regularities to odontocetes remains questionable in many respects. Nonetheless, published studies of TTS in odontocetes allow us to deduce some trends.

Fatiguing sound level and duration

Among the earliest observations, TTS in odontocetes exhibited dependence on both fatiguing noise level and duration. Short noise bursts or sound pulses were slightly effective at moderate or even relatively high levels. High-level (~200 dB re. 1 μPa) and short (1 s) noise bursts have been found to produce noticeable but small (a few dB) and rapidly recovering TTS in both bottlenose dolphins, *Tursiops truncatus*, and beluga whales, *Delphinapterus leucas* (Schlundt et al., 2000). Even higher-level (up to 210 dB re. 1 μPa) but equally short sound pulses elicited either smaller or insignificant TTS (Finneran et al., 2000) or resulted in small TTS in the same species (Finneran et al., 2002). Similar results were obtained in a study of the harbor porpoise, *Phocoena phocoena*, which demonstrated that attaining the relatively low TTS criterion of 6 dB required short, low-frequency sound pulses of a seismic airgun of at least 200 dB re. 1 μPa (Lucke et al., 2009). Conversely, a lower (179 dB re. 1 μPa) but longer (30–50 min) exposure of low-frequency (4 to 11 kHz) fatiguing noise resulted in definite TTS (up to 11 dB) in *T. truncatus* (Nachtigall et al., 2003).

Because the sound pressure level (SPL) and the duration of the fatiguing noise can both influence the TTS, fatiguing noise has been characterized by its sound exposure level (SEL) in many investigations. SEL is a dB measure of the temporal integral of the squared sound pressure (specified as dB re. 1 $\mu Pa^2 s$); when the sound pressure is kept constant during the exposure, the SEL is simply a dB measure of the product of the squared sound pressure and the exposure duration. This parameter is an equivalent of a dB measure of the overall sound energy flux density ($J m^{-2}$), which uses the squared sound pressure (Pa^2) as a variable that is proportional to the power flux density ($W m^{-2}$). Finneran et al. (Finneran et al., 2005) suggested a threshold of 195 dB re. 1 $\mu Pa^2 s$ to trigger TTS in *T. truncatus*.

The consideration of the SEL index alone, without the additional specification of SPL and duration, is valid only in cases of complete time–intensity trading (i.e. when noises of equal SELs but of different level-to-duration ratios produce equal TTSs). This, however, is not always the case. Finneran et al. (Finneran et al., 2010a) have demonstrated a substantial increase in TTS with increasing exposure duration when the SEL is kept constant. Mooney et al. (Mooney et al., 2009) reported a similar correlation. At other conditions, the relationship might be the opposite: a study of the finless porpoise, *Neophocaena phocaenoides*, has demonstrated higher level-dependent than duration-dependent TTS growth. In any case, unequal level-dependent and duration-dependent TTS growth indicates that not only SEL but also the level-to-duration ratio determines the efficiency of fatiguing noise.

To quantitatively describe the correlation between fatiguing sound level and duration, Finneran et al. (Finneran et al., 2010a) suggested that TTS is the product of the function of TTS *versus* level and the function of TTS *versus* exposure duration. Qualitatively, it is the same manner of dependence that is known in terrestrial mammals (Carder and Miller, 1972; Clark, 1991). It implies that both the level-dependent TTS growth varies depending on the exposure duration and that duration-dependent growth varies depending on the sound level.

TTS timing

An important characteristic of TTS is its timing, i.e. how quickly or slowly it arises during the fatiguing noise and recovers after the noise cessation. Some data regarding TTS timing in odontocetes have been obtained using both the behavioral and electrophysiological methods. Unlike many investigations in humans and laboratory mammals that have traced TTS growth and recovery for hours to days or months (Melnick, 1991; Clark, 1991), investigations in cetaceans have mostly focused on short-term recovery (minutes to hours). These investigations have demonstrated variable durations of TTS recovery, depending on the experimental conditions and initial TTS value. In the first evoked-potential TTS investigation in *T. truncatus* (30 min noise exposure of 4–11 kHz bandwidth, 160 dB re. 1 μPa), a TTS of 8 dB at 5 min after exposure (the earliest time point) recovered at a rate of ~1.5 dB per time doubling, completing recovery in less than 1 h (Nachtigall et al., 2004). A higher TTS (up to 40–45 dB) revealed by the evoked-potential method after exposure to a 20 kHz tone of 186–194 dB re. 1 μPa (203–206 dB re. 1 $\mu Pa^2 s$) resulted in almost no recovery within the first hour after exposure, requiring up to 4 days for a complete recovery (Finneran et al., 2007). A study of TTS growth and recovery in *T. truncatus* after exposure to a 3 kHz tone has demonstrated TTS dependence on the initial value: the higher the TTS, the steeper the post-exposure recovery *versus* time function, although at a high TTS (more than 20 dB), the recovery was incomplete after 30 min of observation time (Finneran et al., 2010a). Finneran et al. (Finneran et al., 2010b) compared the effects of intermittent sound exposure (four exposures, each 16 s long with a duty cycle of ~7%) with continuous exposure of the same overall duration (64 s). The intermittent exposure resulted in a lower final TTS than continuous exposure, thus revealing a partial recovery during the pauses of intermittent exposure. These results suggest the possibility of partial recovery during the long exposure itself.

Across-frequency spread of TTS

Behavioral measurements in laboratory mammals have shown that, in general, maximum TTS occurred at frequencies ~0.5 octaves above the center frequency of the exposure band (Clark, 1991). Several investigations in odontocetes have confirmed this regularity. Despite the variety of experimental conditions, the investigations have indicated that the highest TTS appeared at test frequencies above the fatiguing sound frequency, roughly 0.5 to 1 octave (Schlundt et al., 2000; Nachtigall et al., 2004; Finneran et al., 2007; Lucke et al., 2009; Mooney et al., 2009; Popov et al., 2011).

The dependence of TTS on fatiguing sound frequency and bandwidth

A wide variety of fatiguing sounds has been used in different investigations: short wide-band pulses imitating distant explosions (Finneran et al., 2000) or seismic airgun impulses (Finneran et al., 2002; Lucke et al., 2009), band-passed noise (Nachtigall et al., 2003; Nachtigall et al., 2004; Mooney et al., 2009; Popov et al., 2011) and pure tones of various frequencies (Schlundt et al., 2000; Finneran et al., 2007; Finneran et al., 2010a; Finneran et al., 2010b).

The majority of the investigations used only one type of fatiguing sound and therefore could not demonstrate any dependence of TTS on the frequency of the fatiguing sound. A variety of fatiguing pure tones was tested by Schlundt et al. (Schlundt et al., 2000) in *T. truncatus* and *D. leucas*; however, the TTS effect obtained was minor, and the authors did not report any frequency-dependent regularity. Later, combining the data obtained from different studies, Finneran and Schlundt (Finneran and Schlundt, 2010) concluded that a 20 kHz fatiguing tone more effectively produces TTS in *T. truncatus* than a 3 kHz tone (i.e. the higher the fatiguing sound frequency, the higher the TTS). Popov et al. (Popov et al., 2011) observed an opposing regularity in *N. phocaenoides*: the strongest noise effect (both the highest TTS and the longest recovery) was observed for the 22.5 kHz noise and 32 kHz probe, whereas the least significant effect was observed at the 90 kHz noise and 128 kHz probe (i.e. the lower the frequency, the greater the effect). Taken together, the dependence of fatiguing sound effects on frequency requires more detailed investigation.

Summarizing the data reviewed above, we suggest that there are several issues that require more detailed investigation: (1) the dependence of TTS on fatiguing sound level and duration; (2) the tracing of the temporal dynamics (growth and recovery) of TTS; and (3) the dependence of TTS on fatiguing sound frequency and bandwidth. The goal of this study was to obtain more data regarding these issues.

MATERIALS AND METHODS

Subjects and experimental facilities

The study was conducted at facilities of the Utrish Marine Station of the Russian Academy of Sciences (the Black Sea coast). The subjects were two young beluga whales, *Delphinapterus leucas* (Pallas 1776), including a 2-year-old male (body length 264 cm, body mass 270 kg) and a 2-year-old female (body length 240 cm, body mass 250 kg). The animals were housed in a 9×4×1.2 m pool filled with seawater. The care and use of the animals were in compliance with the Guidelines of the Russian Ministry of Higher Education on the use of animals in biomedical research.

During the experiments, the animals were removed from their home pools and placed on a stretcher in a small 4.5×0.85×0.6 m wooden tank filled with seawater in such a manner that the dorsal surface of the head with the blowhole remained above the water surface. For non-invasive evoked-potential recording, suction-cup electrodes were applied that consisted of a 15 mm stainless-steel disk mounted within a 60 mm silicon suction cup. The active electrode was fixed at the vertex head surface, 7 cm behind the blowhole above the water surface. The reference electrode was fixed at the back. The electrodes were connected by shielded cables to the input of a custom-made EEG amplifier based on an AD-620 chip and isolation AD-202 unit (Analog Devices, Norwood, MA,

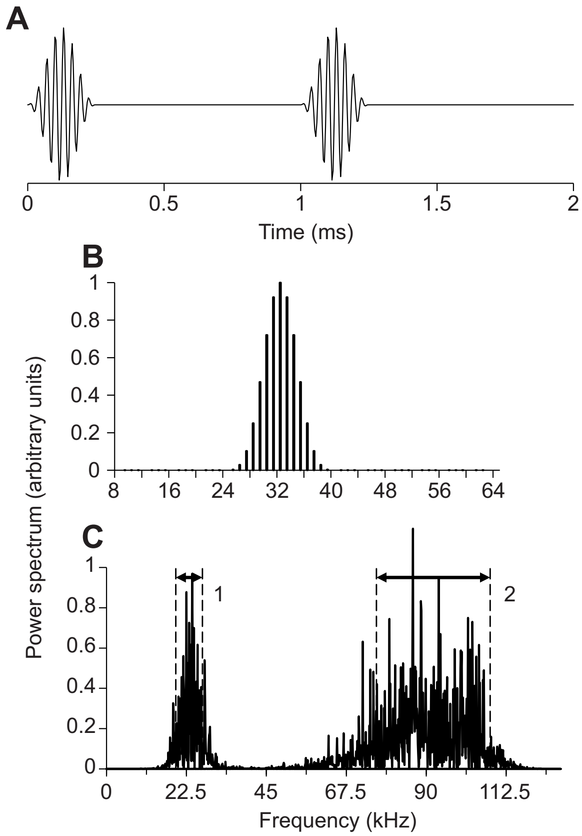

Fig. 1. An example of the probe signal waveform (A) and spectrum (B). A signal of a carrier frequency of 32 kHz is shown. For better resolution, only an initial part containing two pips of the 16 pip train is presented in A. (C) Frequency spectra of fatiguing noises of 22.5 kHz (1) and 90 kHz center frequencies (2). Spectra of 8 s noise samples are presented overlapping on the common frequency scale; vertical dashed lines and double-headed arrows delimit nominal frequency bands (19.5 to 27 kHz for the 22.5 kHz center frequency, 76 to 108 kHz for the 90 kHz center frequency).

USA) that provided 80 dB gain within a frequency range of 200 to 5000 Hz. The amplified signal was digitized and collected using a DAQ-6062E data acquisition card (National Instruments, Austin, TX, USA) and stored in computer memory.

Test and fatiguing sounds

The test sound stimuli were trains of tone pips. Each train was 16 ms long and contained 16 pips at a rate of 1000 pips s^{-1}. The carrier frequencies of the pips varied from 8 to 128 kHz. Each pip of the train, irrespective of the carrier frequency, contained eight cycles of the carrier enveloped by a cosine function (Fig. 1A). Thus, pip durations varied from 62.5 μs at a carrier frequency of 128 kHz to 1 ms at a carrier frequency of 8 kHz. The frequency spectra of such pips (Fig. 1B) were 0.25 octaves wide at a level of 0.5 of the power peak (–3 dB); their equivalent rectangular bandwidth was 0.32 octaves. This sort of test stimulus was used because it more effectively produced the rhythmic auditory evoked response [the envelope following response (EFR)] than a narrow-band sinusoidally modulated tone (Supin and Popov, 2007). The stimulus bandwidth was sufficiently narrow to assign a resulting measurement to a particular sound frequency with a tolerance of ~0.25 octaves. The pip trains were presented at a rate of 16 trains s^{-1}. The SPL of the pip trains was specified in dB re 1 μPa of root mean square (r.m.s.) sound pressure over the 16 ms pip-train duration.

The fatiguing noise was half-octave band-filtered noise (second-order Butterworth filter) with center frequencies from 11.2 to 90 kHz (Fig. 1C). The SPL of the noise was specified in dB re. 1 μPa of r.m.s. sound pressure.

Both the test and fatiguing signals were digitally synthesized at an update rate of 512 kHz and were digital-to-analog converted by the same DAQ-6062E card, amplified, attenuated and played using either an ITC-1032 transducer (International Transducer Corporation, Santa Barbara, CA, USA) for frequencies of 8–45 kHz or a B&K 8104 transducer (Bruel & Kjær, Nærum, Denmark) for frequencies of 64–128 kHz. The transducer was positioned at a distance of 1 m in front of the animal's head, proximal to the front wall of the tank. The playback channel was calibrated prior to and after the experiments by positioning a calibrated receiving hydrophone (B&K 8103) near the animal's head. Sound monitoring revealed that, despite the sound reflections within the tank, local sound levels around the animal's head varied within a range of less than 5 dB.

Evoked-potential recording and threshold determination

For recording of the evoked-potential response, 25 ms sweeps that were synchronous with the test stimuli presentation were extracted from the brain-potential records. To extract the signal from noise, the sweeps were coherently averaged on-line using triggering from the stimulus onset. For further analysis, a 16 ms segment of the averaged record (from the fifth to the 21st ms relative to the stimulus onset) containing a rhythmic evoked-potential response (EFR) to the pip-train stimulus was Fourier transformed on-line to obtain the response frequency spectrum. The magnitude of the 1 kHz spectral peak was considered to be a measure of the response magnitude.

To trace both the pre- and post-exposure threshold dynamics, an adaptive one-up–one-down (staircase) procedure of stimulus variation was used. This procedure required on-line decisions concerning the presence or absence of the response to be made. To make these decisions, an arbitrary criterion was applied: a record was considered to be response-present when the 1 kHz peak in the response spectrum was more than twice as high as any of spectrum components within an adjacent range from 0.75 to 1.25 kHz. On-line averaging was continued until either the response-present criterion was achieved (this trial was estimated as response-present) or the response-present criterion was not achieved, but all spectral components within the range of 0.75 to 1.25 kHz were below 0.01 μV r.m.s. (this trial was estimated as response-absent). With this rule applied, 100 to 500 traces were generally averaged to collect one record. With the test signal presentation rate of 16 trains s^{-1}, each record required ~6 to 31 s. Stimulus levels were varied by 5 dB increments/decrements. If the response was detected according to the criterion specified above, the next stimulus level was decreased by 5 dB; if the response was absent, the next stimulus level was increased by 5 dB. Reversal points (transitions from stimulus level increase to decrease and *vice versa*) were selected, and the middle point of each pair of adjacent reversal points (the local maximum and minimum) was assigned as an instant threshold estimate attributed to the middle point of the two corresponding time instants.

Additionally, at the beginning of the study, baseline thresholds were measured using a method based on EFR amplitude dependence on stimulus levels within a wider level range. The EFR was recorded at a variety of probe levels, from provisionally sub-threshold (no 1 kHz response peak visible in the response frequency spectrum) to 25–30 dB above the anticipated threshold. The response peak magnitudes were plotted as a function of the probe levels. This function was approximated by a straight regression line, which was extrapolated to the zero-response amplitude; the resulting test-sound level was considered to be the baseline threshold estimate (Supin and Popov, 2007).

Fatiguing noise exposure and threshold tracing conditions

The fatiguing noise level was 165 dB re. 1 μPa, and the exposure durations were 1, 3, 10 or 30 min. During each experimental session, the fatiguing noise was presented either once or twice. If two exposures were presented during a session, the second exposure was 10 times longer than the first one (i.e. 10 min after a 1 min exposure or 30 min after a 3 min exposure) and was presented at 30 min after the first post-exposure tracing. We assumed that this protocol was acceptable because apart from recovery after the first exposure, the total exposure duration increased by no more than 10% of the second exposure duration.

Post-exposure thresholds were traced for no longer than 1 h, even if the total recovery was not achieved. This limit was applied to restrict the amount of time that the animal subject was kept in the tank during the experiment. The longest session time included two noise exposures: two 1 h post-exposure tracings, and a 30 min pause before the second exposure, thus ~3 h. By this limiting the total treatment time, no disturbances in the animal's behavior were observed upon its return to the home pool.

RESULTS

Response features

Typical evoked responses to the pip-train probe signals recorded in the belugas, together with their frequency spectra, are presented in Fig. 2A,B. The pip train evoked EFR (i.e. a sequence of evoked potential waves following the pip rate). Both the beginning and the end of the EFR featured a lag of ~5 ms relative to the stimulus. This lag reflects the evoked-response latency that confirms the physiological but not the artifactual origin of the recorded waveforms. The response was level-dependent: a decrease in stimulus level resulted in a decrease in EFR amplitude until the response disappeared in noise (at a level of 55 dB re. 1 μPa in the exemplified case).

The frequency spectra of the records featured a definite peak at the frequency equal to the stimulus pip rate of 1 kHz (Fig. 2B). The response magnitude *versus* probe level functions (shown in Fig. 2C) were satisfactorily approximated by straight regression lines (r^2=0.95 to 0.99; in Fig. 2, r^2=0.98). At near-threshold probe levels, the transition from a present to absent response (according to the criteria specified above) appeared within one 5 dB decrement, as exemplified by the 60 and 55 dB levels shown in Fig. 2.

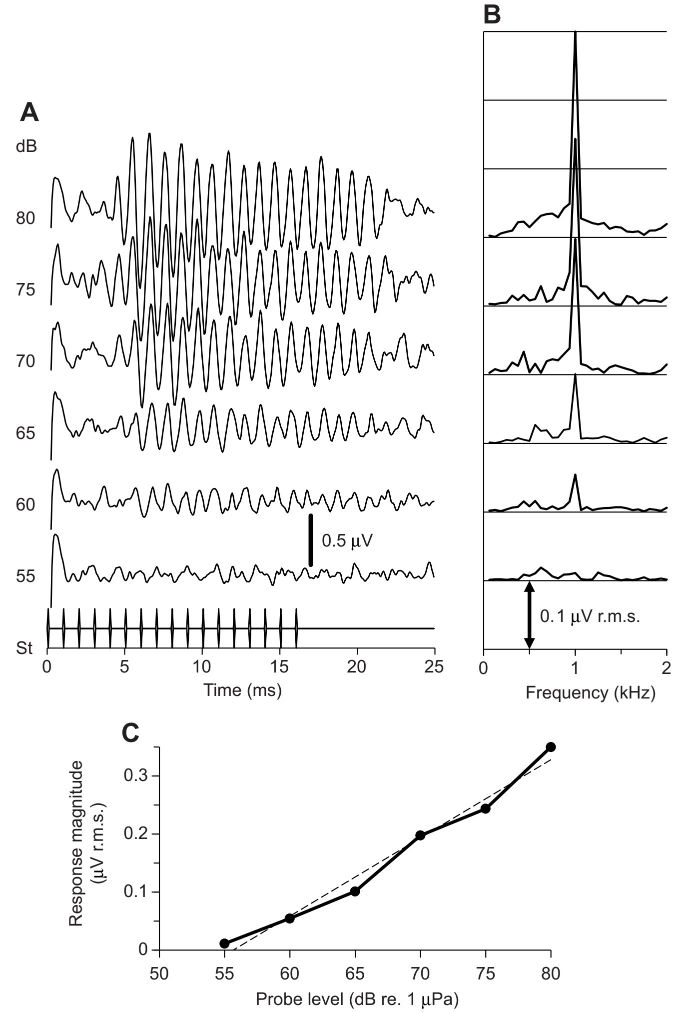

Fig. 2. Representative examples of envelope following response (EFR) waveforms (A) and spectra (B). Probe levels (dB re. 1 μPa) are specified near the records. St, the test stimulus envelope. (C) EFR magnitude dependence on probe level. The straight dashed line indicates the approximation of the magnitude *versus* level function by a regression line.

Baseline and pre-exposure thresholds

Before starting the exposure program, the baseline thresholds were measured in both of the subjects using the regression-line threshold evaluation technique. Complete audiograms with 0.25 octave frequency steps were obtained (Fig. 3). In both of the subjects, the lowest thresholds (46 to 52 dB re. 1 μPa) were detected at frequencies ranging from 32 to 90 kHz. The differences between the thresholds in the two subjects were not more than 5 dB.

In each session, the probe frequency threshold was re-measured before the noise exposure using the staircase procedure. For the majority of the reversals, the span between the adjacent maximum and minimum was within one 5 dB step; rarely there were spans of two steps (10 dB). The mean of six pre-exposure instant threshold estimates was taken as a pre-exposure threshold. Limited data on threshold variability were extracted from these pre-exposure threshold estimates, as presented in Fig. 4 for the test frequencies of 16, 32, 64 and 128 kHz. The session-by-session pre-exposure variations were less than ±5 dB relative to either the baseline (regression-line estimated) threshold or the across-session mean of pre-exposure thresholds, with standard deviations from 1.8 dB (male, 64 kHz) to 3.8 dB (male, 32 kHz). Differences between the baseline threshold estimates and means of the pre-exposure threshold estimates varied from 0.3 dB (male, 32 kHz) to 3.5 dB (female, 128 kHz).

Limited availability of the subjects did not allow us to trace threshold variability during control (no-exposure) sessions.

Post-exposure threshold tracing

The staircase post-exposure threshold tracing is exemplified in Fig. 5.The probe levels were shifted up and down by 5 dB steps depending on the present or absent responses according to the criteria specified above (Fig. 5A). For the majority of the reversals, the span between the adjacent maximum and minimum was within one 5 dB step. Occasionally there were spans of two steps (10 dB), and in rare cases, three steps (15 dB, one pair in Fig. 5A). From this

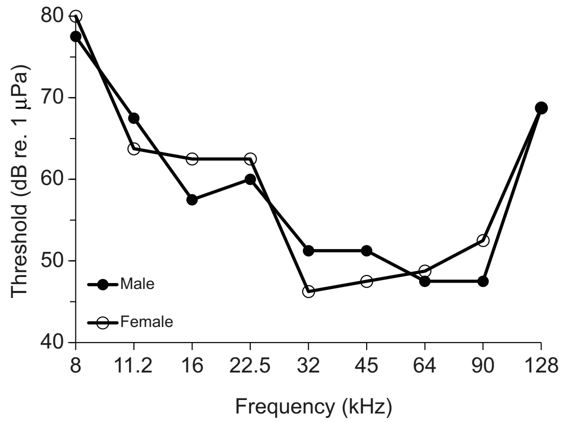

Fig. 3. Baseline audiograms of the two subjects.

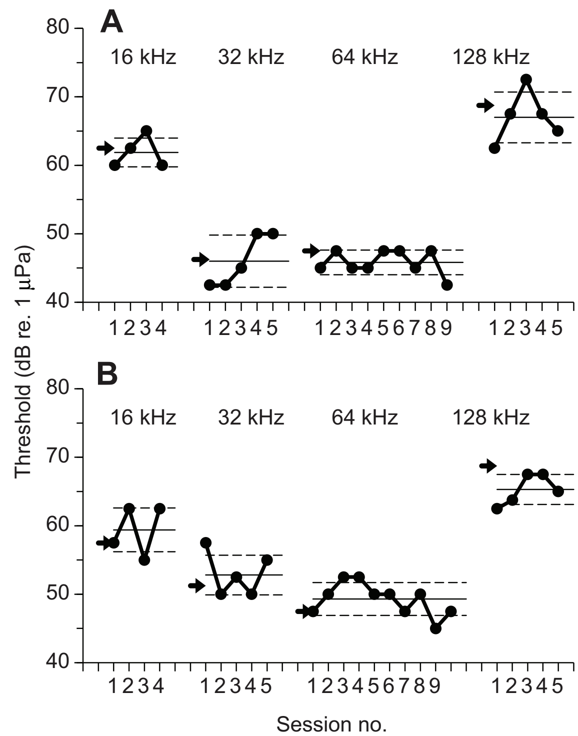

Fig. 4. Inter-session variability of the pre-exposure threshold estimates. Each of the plots shows pre-exposure threshold estimates in four to 10 sessions where the particular probe frequency, from 16 to 128 kHz, was tested. Solid and dashed straight lines show the mean and standard deviation for each of the plot, respectively; arrows show the baseline threshold estimates according to Fig. 3. (A) Male. (B) Female.

sequence, reversal points (local maxima and minima of the test levels) were selected, excluding intermediate levels. The middle points of the reversal pairs were considered to be threshold estimates that were plotted as a function of time. The differences between the instant and the pre-exposure thresholds were considered as the instant TTS (Fig. 5B). TTS at 1.5 min post-exposure ($TTS_{1.5}$) is marked in Fig. 5B; in the presented case, it was 47.5 dB.

Either immediately after the end of the exposure or after a certain delay (plateau), threshold recovery was observed. To separate the two fractions of the TTS *versus* time function, the plateau and recovery phases, we used an arbitrary criterion: a part of the function for which the thresholds were not below 5 dB re. maximum was considered to be a plateau. A fraction of the function after the plateau was considered to be the threshold recovery phase. The recovery was considered to be complete when the thresholds were no more than 5 dB above the pre-exposure level. The 5 dB tolerance was applied to account for possible imprecision of the threshold evaluation.

The plateau was assumed to have no trend by definition. It was approximated by a flat straight line that was specified by its mean level (dB). The recovery phase featuring a trend was approximated by a logarithmic regression line that was specified by its starting-point level (dB) and trend (dB per log unit). The starting point of the regression line was either the end of the plateau or (in the absence of a plateau) a 1.5 min post-exposure point (the earliest point available in all post-exposure tracings).

Using these statistical parameters, two characteristics of the post-exposure function were derived: (1) initial post-exposure TTS ($TTS_{1.5}$), the difference between the plateau or 1.5 min starting point of the regression line and the pre-exposure threshold; and (2) TTS duration (D_{TTS}), the time when the regression line reached the pre-exposure threshold value. In the example presented in Fig. 5, $TTS_{1.5}$ was evaluated as 47.5 dB and D_{TTS} as 41 min. In cases when thresholds did not reach the pre-exposure level during the observation time of 60 min, the regression line was extrapolated, but not beyond 120 min, assuming that the longer the extrapolation, the more it is error-prone. When the extrapolated regression line did not reach the pre-exposure level before 120 min, D_{TTS} was specified as '>120 min'.

TTS manifestation in post-exposure audiograms

The impact of fatiguing noise is shown in Figs 6 and 7 using a constant noise duration (10 min) but at different noise center frequencies (from 11.2 to 90 kHz with 1 octave steps). For each of these noise versions, the respective TTS was traced at the following probe frequencies: (1) 0.5 octave below the noise center frequency, (2) at the noise frequency, and (3–5) 0.5, 1 and 1.5 octave above the noise frequency. For brevity, these probe frequencies are referred to as –0.5, 0, +0.5, +1 and +1.5 octave, respectively. Thus, for each of the noise versions, a part of the post-exposure audiogram (from –0.5 to +1.5 octave at half-octave steps) was obtained at various post-exposure times. The only exception was the 90 kHz noise, for which frequencies ranging from –0.5 to +0.5 octave were tested because the +1 and +1.5 octave probes (180 and 256 kHz, respectively) would exceed the auditory frequency range.

The resulting post-exposure audiograms at different post-exposure time points (1.5, 5, 15, 30 and 60 min) are shown in Figs 6 and 7 (for both the male and female, respectively), together with the baseline audiogram. The figures demonstrate that, after the exposure, an audiogram trough (threshold increase) appeared. In the majority of cases, the threshold trough extended from –0.5 to +1.5 octave relative to the noise center frequency, with the deepest point (the highest threshold) at +0.5 octave. In some instances (the female, 11.2 and 22.5 kHz noise), the trough was wider. The depth of the trough varied from a few dB (male, 90 kHz noise) to 62.5 dB (female, 22.5 kHz noise).

TTS recovery time course

As shown in Figs 6 and 7, after the initial post-exposure increase, the thresholds gradually restored, i.e. TTS recovered. Shown in Fig. 8

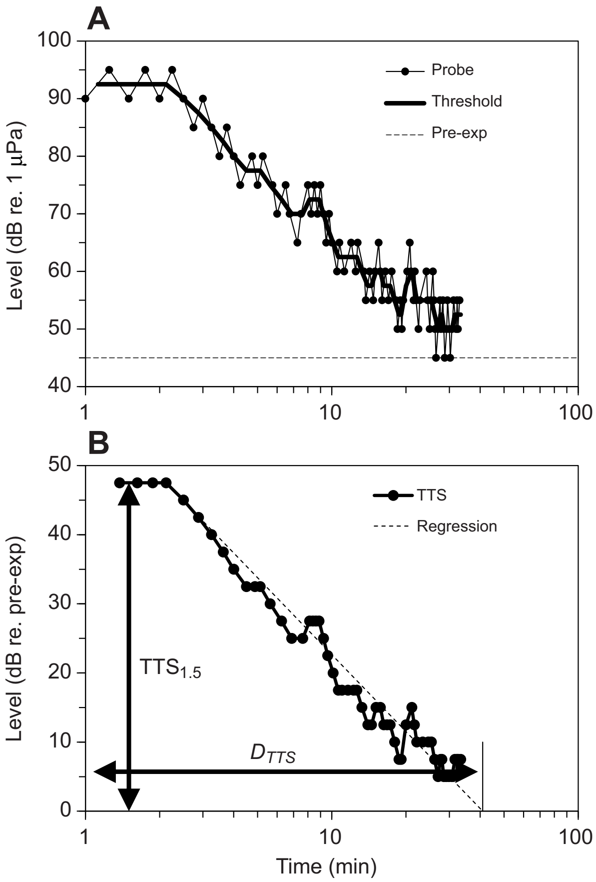

Fig. 5. An example of the post-exposure threshold tracing and the temporary threshold shift (TTS) effect estimates (male, 45 kHz fatiguing noise, 10 min exposure, 64 kHz probe frequency). (A) Probe levels varied according to the staircase procedure (probe) and threshold *versus* time function (threshold); horizontal dashed line indicates the pre-exposure threshold (pre-exp). (B) TTS *versus* time function derived from A. Dashed line indicates the approximation of the recovery fraction of the TTS *versus* time function by a log regression line. Arrows mark initial post-exposure TTS ($TTS_{1.5}$) and TTS duration (D_{TTS}).

are the TTS *versus* time functions at all of the tested noise center frequencies (from 11.2 to 90 kHz), at the +0.5 octave (the most TTS-sensitive) test frequency.

Both the plateau and recovery phases of the TTS *versus* time fractions varied over a wide range depending on both the subject (the male or female) and the fatiguing noise frequency. In the male subject, the highest $TTS_{1.5}$ was 47.5 dB (22.5 kHz noise) and the lowest $TTS_{1.5}$ was 10.5 dB (90 kHz noise); in the female subject, $TTS_{1.5}$ at the same noise frequencies varied from 27.5 to 62.5 dB. The shortest plateau was less than 2 min, i.e. virtually absent (Fig. 8, male, 11.2 kHz noise), whereas the longest observed plateau was as long as 15 min (female, 22.5 kHz noise). The regression lines approximating the recovery phase featured the slope from 16.1 dB per time log unit (female, 90 kHz noise) to 36.3 dB per time log unit (male, 22.5 kHz noise). The overall recovery duration D_{TTS} (the plateau plus recovery phase) varied in the male subject from 13 min (90 kHz noise) to 41 min (22.5 kHz noise) and in the female subject, as estimated by extrapolation of regression lines, from 120 min (90 kHz noise) to longer than 120 min (11.2 to 45 kHz noise). However, the recovery was never longer than 24 h (the next day session test).

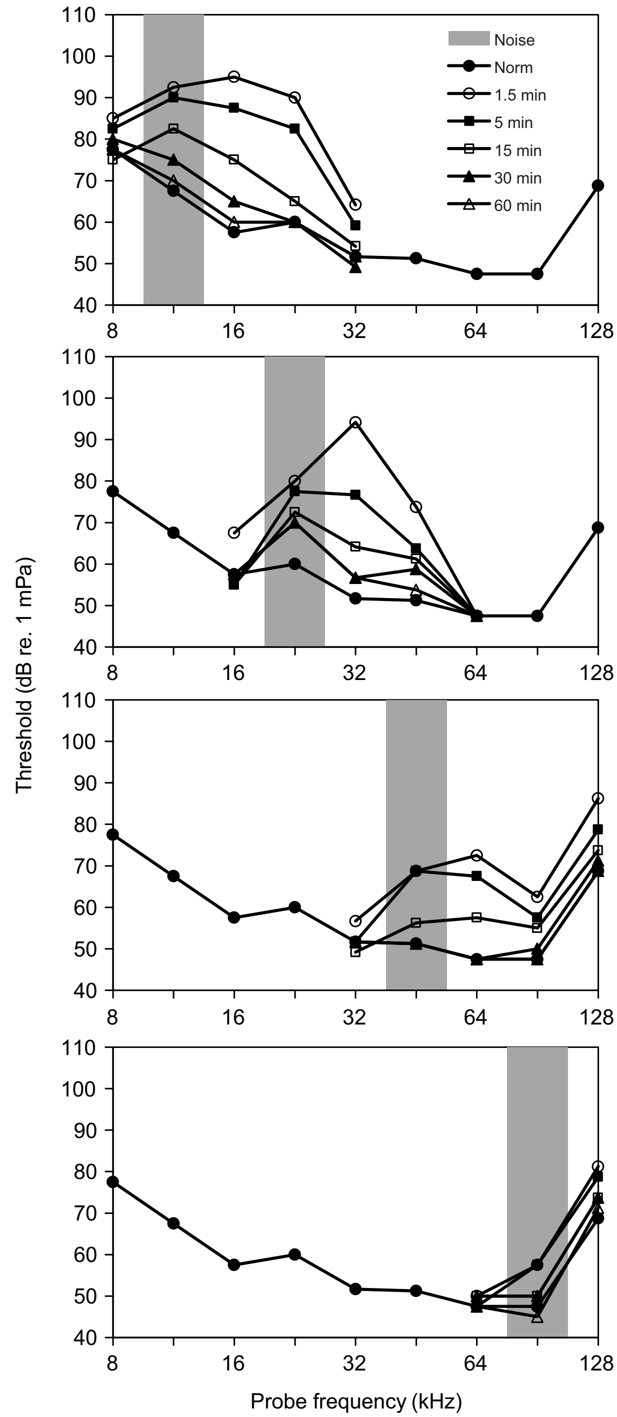

Fig. 6. Background and post-exposure (half-octave noise of 165 dB re. 1 µPa, 10 min) audiograms of the male subject. The shaded bars mark the noise bandwidth (center frequencies of 11.2, 22.5, 45 and 90 kHz). The post-exposure audiograms were obtained from 1.5 to 60 min after the end of exposure, as indicated.

Dependence of TTS on noise and probe frequency

The results of $TTS_{1.5}$ and D_{TTS} measurements at various noise and probe frequencies are presented in Table 1 and Fig. 9. From these results, the tendencies were derived as follows:

1. The highest $TTS_{1.5}$, as well as the longest D_{TTS}, appeared at a test frequency above the noise frequency. In the majority of cases,

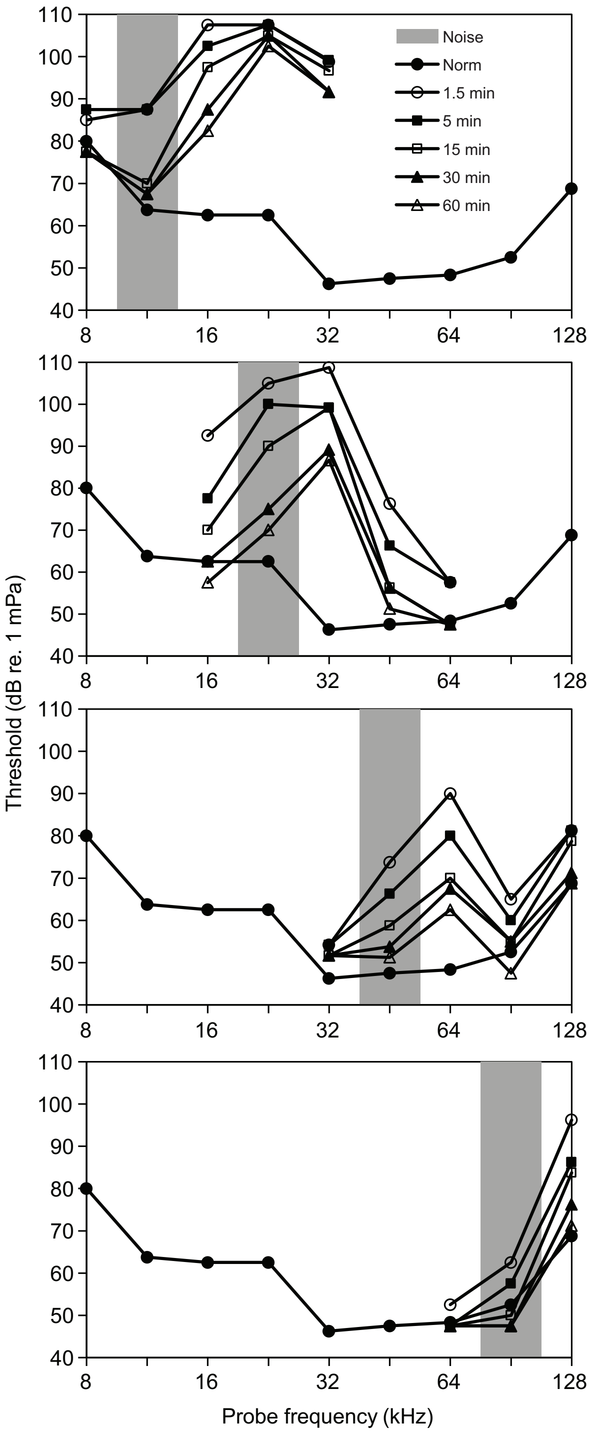

Fig. 7. As Fig. 6, but for the female.

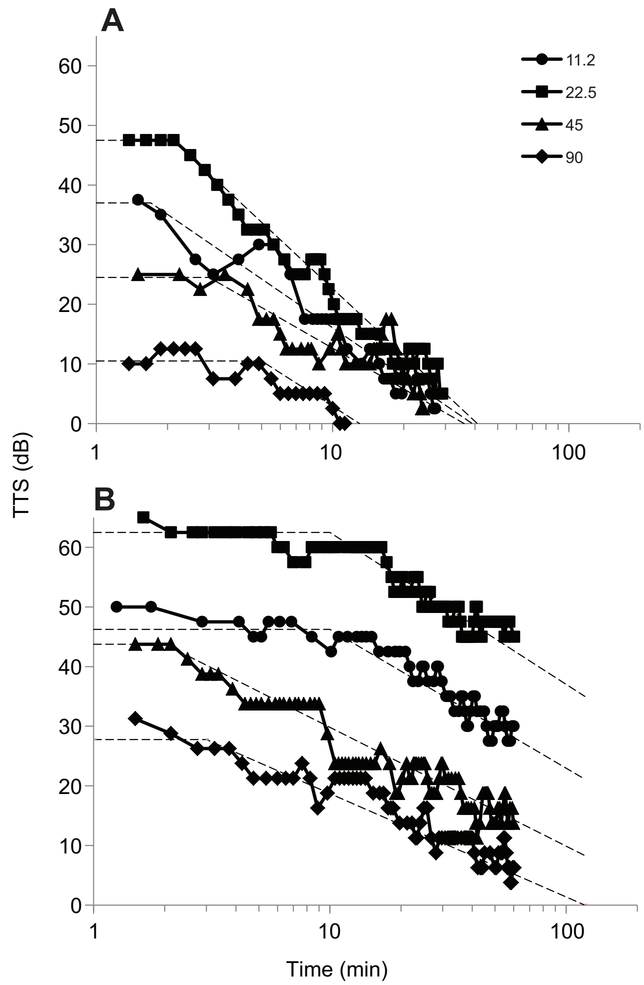

Fig. 8. TTS *versus* time functions after a 10 min exposure to noise of various center frequencies, from 11.2 to 90 kHz. (A) Male; (B) female. Dashed lines: the approximations of the function; flat segment: plateau; oblique segment: recovery phase.

it was a probe frequency of +0.5 octave. At this probe frequency, the maximum $TTS_{1.5}$ was as high as 47.5 and 62.5 dB in the male and female subjects, respectively (Fig. 9A,B, 22.5 kHz noise). The D_{TTS} was as long as 60 min and >120 min in the male and female subjects, respectively. The only exception was the exposure of the female subject to a 11.2 kHz noise. For this noise version, the highest $TTS_{1.5}$ (55 dB) was at a probe frequency of +1.5 octave.

2. The effects of noise depended on the noise center frequency. Among the four noise versions tested, the most effective version was the 22.5 kHz noise. At the +0.5 octave probe frequency, it produced a $TTS_{1.5}$ of 45 and 62.5 dB and a D_{TTS} of 60 and >120 min in the male and female subjects, respectively. The 11.2 kHz noise was also very effective in the female subject; at probe frequencies of +1 and +1.5 octave, its effects were comparable with those of the 22.5 kHz noise. At noise frequencies above 22.5 kHz (45 and 90 kHz), both the $TTS_{1.5}$ and D_{TTS} decreased.

3. There was a considerable difference in the noise effects between the two subjects. As mentioned above, the most effective 22.5 kHz noise produced a $TTS_{1.5}$ of 45 dB in the male and 62.5 dB in the female, i.e. the difference was 17.5 dB. The D_{TTS} produced by the same noise (50 and >120 min in the male and female, respectively) also differed markedly.

Dependence of TTS on exposure duration

Similarly to the previous series of measurements, in this series the center frequencies of the fatiguing noise were 11.2, 22.5, 45 and 90 kHz. However, the TTS effects were measured at a constant test frequency of +0.5 octave (the most effective frequency) and at different exposure durations: 1, 3, 10 and 30 min. Exceptions were exposures at 11.2 and 22.5 kHz noises in the female subject because the 10 min exposure produced a very high TTS. Therefore, to

Table 1. Dependence of the temporary threshold shift (TTS) on noise center frequency (11.2 to 90 kHz) and test frequency (–0.5 to 1.5 octaves relative to the noise center frequency)

Probe frequency (octaves re. noise center)	11.2 kHz		22.5 kHz		45 kHz		90 kHz	
	Male	Female	Male	Female	Male	Female	Male	Female
–0.5	5	15	7.5	40	7.5	7.5	3.8	8.8
	8	10	4	60	5	4	3	5
0	27.5	27.5	20	52.5	23.8	20	5	20
	33	35	25	>120	35	59	11	18
0.5	37.5	50	47.5	62.5	25	43.8	10	31.3
	41	>120	50	>120	60	>120	30	120
1	30	47.5	26.8	27.5	12.5	22.5	–	–
	25	>120	45	50	32	105		
1.5	17.5	55	5	13.8	12.5	11.3	–	–
	8	>120	8	15	13	8		

10-min exposure. In each cell, the upper line is the initial post-exposure TTS, $TTS_{1.5}$ (dB), and the lower line is the TTS duration, D_{TTS} (min).

maintain animal safety, the 30 min exposure was not used. The results are presented in Table 2 and Fig. 10.

At all noise frequencies, the increase of the exposure duration resulted in monotonous increases of the noise effect, in terms of both $TTS_{1.5}$ and D_{TTS}. With the duration increase from 1 to 30 min, which corresponded to the SEL increase by 15 dB, the increase in $TTS_{1.5}$ ranged from 16.25 dB (from 7.5 to 23.75 dB in the male at the 90 kHz noise) to 32.5 dB (from 15 to 47.5 dB in the male at the 45 kHz noise); in series with noise prolongation from 1 to 10 min (SEL increase by 10 dB), the increase in $TTS_{1.5}$ ranged from 25 dB (from 37.5 to 62.5 dB in the female at the 22.5 kHz noise) to 41.75 dB (from 8.75 to 50 dB in the female at the 11.2 kHz noise). Thus, in all the cases, the TTS *versus* duration growth was more than 1 dB/dB. D_{TTS} grew also at a rate higher than 1 min/min. In the male subject, the growth was from 2.6 min/min (at the 22.5 kHz noise) to 3.8 min/min (at the 90 kHz noise) within an exposure duration range from 1 to 10 min and was not measurable at 30 min exposure because of long recovery time; in the female subject, the growth was as high as 11 min/min at the 90 kHz noise within a range from 1 to 10 min and was not measurable at other noise frequencies because of long recovery time.

DISCUSSION

Data representativeness

A methodical restriction inevitably admitted in the present study was that only two subjects were available for a limited time, with only one measurement performed at each of the fatiguing noise and test sound combinations. Repeating the threshold tracings at each of the exposure conditions and without exposure in order to assess possible variations was not possible. Therefore, the results obtained herein cannot be generalized quantitatively. However, some qualitative tendencies can be derived.

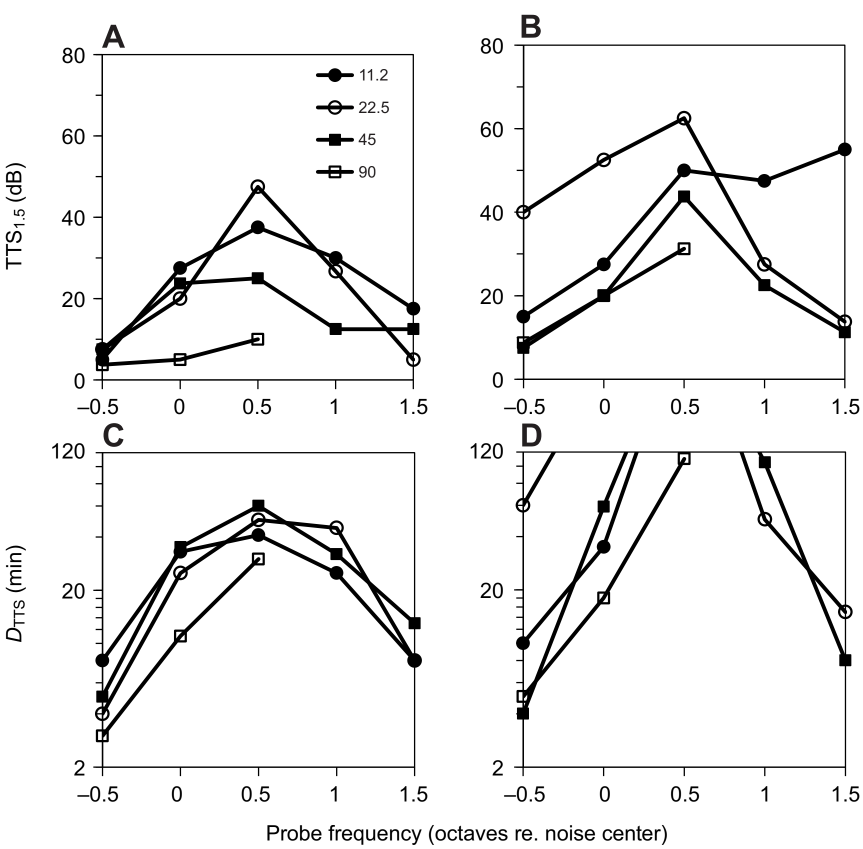

Fig. 9. Dependence of noise effects on the noise and test frequencies. (A,B) $TTS_{1.5}$, (C,D) D_{TTS}; (A,C) male; (B,D) female. The noise center frequencies (11.2 to 90 kHz) are indicated in the key. Plots directed outside the upper boundary of the panel symbolize D_{TTS} exceeding 120 min.

Table 2. Dependence of the temporary threshold shift (TTS) on the noise center frequency (11.2 to 90 kHz) and exposure duration (1 to 30 min)

Exposure duration (min)	11.2 kHz		22.5 kHz		45 kHz		90 kHz	
	Male	Female	Male	Female	Male	Female	Male	Female
1	15	25	27.5	37.5	12.5	8.8	7.5	21.3
	13	12	19	58	21	31	8	8
3	22.5	27.5	45	57.5	15	28.8	8.8	21.3
	20	35	35	95	32	75	13	30
10	37.5	50	47.5	62.5	25	43.8	10	31.3
	41	>120	50	>120	60	>120	30	110
30	47.5	–	55	–	42.5	51.3	23.8	31.3
	>120		>120		>120	>120	>120	>120

Test frequency=+0.5 octave. In each cell, the upper line is the initial post-exposure TTS, $TTS_{1.5}$ (dB), and the lower line is the TTS duration, D_{TTS} (min).

Inter-individual differences

The experiments revealed a considerable difference of the TTS effects between the two subjects, although the measurements were performed under entirely identical conditions. At a variety of exposure and recording conditions, TTS in the female subject was higher and longer than in the male subject. The difference could not be statistically evaluated at each of the exposure and recording conditions because of the absence of measurement repetitions. However, the general tendency suggested higher susceptibility of the female rather than the male subject to the fatiguing noise.

The limited availability of subjects (only one male and one female subject) does not allow for the establishment of whether this inter-individual difference is gender-associated. Independent of gender, the possibility of notable inter-individual difference must be taken into consideration when the effects of fatiguing sounds on odontocete hearing are assessed.

TTS time course and evaluation of the fatiguing effect

Several previous investigations using either the evoked-potential (Nachtigall et al., 2004) or psychophysical techniques (Finneran et al., 2002; Finneran et al., 2005; Finneran et al., 2010a; Finneran et al., 2010b) have demonstrated more or less fast threshold recovery. Thus, for correct evaluation of fatiguing noise effects, TTS evaluations both as early as possible and as long as possible are important. In the present study, TTS was evaluated as early as at least 1.5 min after the exposure and traced for as long as 1 h. Demonstrated in some experiments, the threshold recovery could begin within the first few minutes of post-exposure. Variation of the fatiguing noise parameters influenced both the immediate post-exposure TTS and the TTS duration.

Across-frequency TTS spread

As mentioned in the Introduction, measurements in laboratory mammals have shown that, in general, maximum TTS occurred at

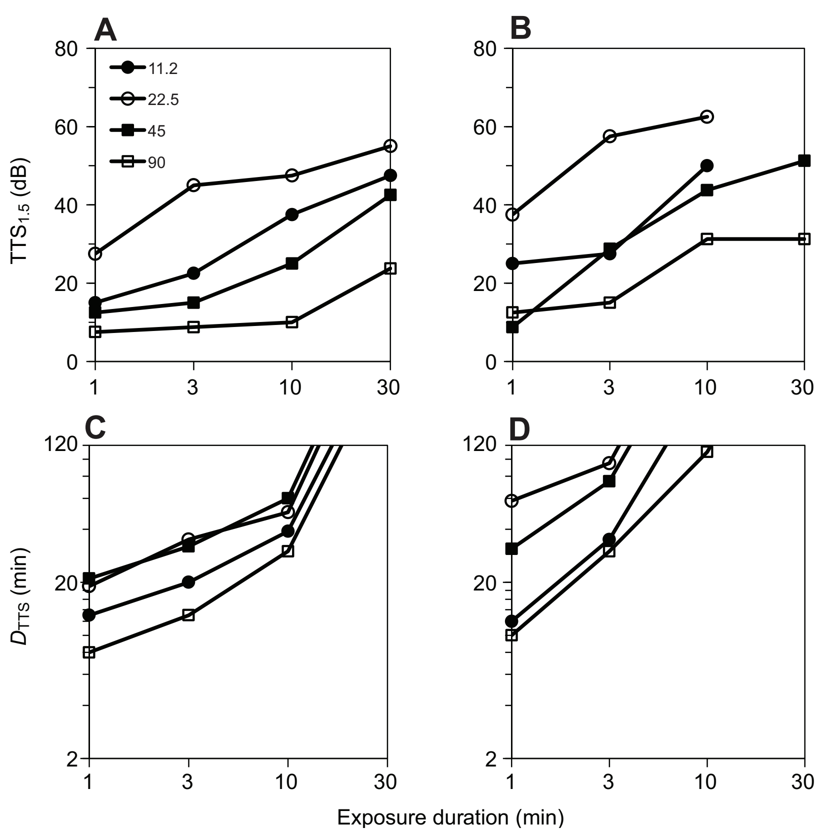

Fig. 10. Dependence of noise effects on the exposure duration. (A,B) $TTS_{1.5}$, (C,D) D_{TTS}; (A,C) male; (B,D) female. The noise center frequencies (11.2 to 90 kHz) are indicated in the key. Plots directed outside the upper boundary of the panel symbolize D_{TTS} exceeding 120 min.

frequencies above the fatiguing sound frequency. The results presented herein suggest that this manner of across-frequency TTS spread in the odontocete subjucts is characteristic of fatiguing sounds within a wide frequency range, starting from 11.2 kHz (which is a relatively low frequency for odontocetes) to the upper boundary of the hearing frequency range. At a majority of the exposure conditions in the present study, a range of across-frequency TTS spread was ~2 octaves, from –0.5 to +1.5 octaves relative to the noise center frequency, with the highest TTS at +0.5 octave. The exception at the 11.2 kHz noise applied to the female (the TTS range shifted ~0.5 octave upward relative to the other cases) does not principally influence the overall trend. Combining the data presented in this study with a variety of previous data allows for the supposition that this or a similar range of TTS across-frequency spread is common for odontocetes.

Dependence of TTS on fatiguing sound frequency

In many previous TTS investigations, only one specific type of fatiguing sound has been evaluated. Combining the data obtained from several studies, Finneran and Schlundt (Finneran and Schlundt, 2010) concluded that a 20 kHz fatiguing tone produced a TTS in *T. truncatus* more effectively than a 3 kHz tone, i.e. the higher the fatiguing sound frequency, the higher the TTS. Popov et al. (Popov et al., 2011) found the highest fatiguing effect for 22.5 kHz noise on the 32 kHz probe and the lowest effect for the 90 kHz noise on the 128 kHz probe in *N. phocaenoides*, i.e. the higher the frequency, the lower the TTS. The results presented herein show that there may be no contradiction between these data. The highest and longest TTS effects were produced by the 22.5 kHz noise. The higher-frequency noises (45 or 90 kHz) produced smaller effects, whereas the lower-frequency noise (11.2 kHz) produced effects either similar to or smaller than those of the 22.5 kHz noise. It is still not proven but it is possible that, within a certain frequency range (around 20 kHz), the auditory system of odontocetes is more susceptible to fatiguing sounds. The acoustical conditions of the experiments presented in this study (the animal's head was near the surface of the water) were not favorable for testing lower frequencies – a point that needs to be addressed in future studies.

Dependence of TTS on fatiguing sound duration

Studies in laboratory mammals (Carder and Miller, 1972; Clark, 1991) have shown that duration-dependent TTS growth depends on the fatiguing sound level. Depending on the level, it may be both lower and higher than 1 dB/dB. Similar relationships are predicted by a model suggested by Finneran et al. (Finneran et al., 2010a) for odontocetes based on data obtained at rather short noise exposures (up to 100 s) and high levels (up to 200 dB re. 1 μPa). At noise levels of around 190–200 dB re. 1 μPa and durations of 64–100 s, the duration-dependent TTS growth was several dB/dB. In this study, the prolongation of fatiguing noise (keeping the noise level constant) resulted in the TTS growth of more than 1 dB/dB at a rather low noise level (165 dB re. 1 μPa) and longer exposures (up to 30 min). Thus, this combination of the noise level and durations still belongs to a range where TTS increased more steeply than the duration-dependent exposed noise energy.

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AUTHOR CONTRIBUTIONS

The study was conceived and designed by V.V.P., A.Ya.S. and V.V.R. Data processing and interpretation, article drafting and revision were carried out by V.V.P. and A.Ya.S. Measurement execution was by D.I.N., E.V.S. V.O.K., M.G.P. and M.B.T. Data processing was conducted by D.I.N. and E.V.S.

COMPETING INTERESTS

No competing interests declared.

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