Conditioned frequency dependent hearing sensitivity reduction in the bottlenosed dolphin (*Tursiops truncatus*)

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Summary

The frequency specificity of conditioned dampening of hearing, when a loud sound is preceded by a warning sound, was investigated in a bottlenose dolphin. The loud sounds were 5-s tones of 16, 22.5, or 32 kHz, SPL of 165 dB rms re 1 μPa. Hearing sensitivity was tested at the same three frequencies. Hearing sensitivity was measured using pip-train test stimuli and auditory evoked potential recording. The test sound stimuli served also as warning sounds. The durations of the warning sounds were varied randomly to avoid locking a conditioning effect to the timing immediately before the loud sound. Hearing thresholds before the loud sound increased, relative to the baseline, at test frequencies equal to or higher than the loud sound frequency. The highest threshold increase appeared at test frequencies of 0.5 octaves above the loud sound frequencies.

Introduction

The negative impact of loud anthropogenic sounds on whales and dolphins manifests itself in a variety of disturbances of their mode of life. These sounds have been associated with the stranding of whales and dolphins (Evans and England, 2001). Strategies to protect whales and dolphins from intense sounds may be either based on considering marine mammals as passive objects (finding them and avoiding or reducing sound exposure) or as active subjects of the mitigation strategy using the avoidance behavior of the animals themselves. In turn, the active avoidance strategies may be based either on behavioral responses (avoiding sound contaminated environments) or specific individual responses like active control of their hearing sensitivity.

Avoidance behavior as a response to loud sounds has long been described in laboratory animals: it was shown that loud sounds can trigger avoidance responses as effectively as other noxious stimuli (Belluzzi and Grossman, 1969). Similar data have been obtained several seal species (Goetz and Janik, 2010). One may assume that loud sounds may be sometimes similarly aversive to whales and dolphins. Many observations in the wild note obviously aversive behavior of whales and dolphins as avoidance of the area of loud man-made sounds (Southall et al. 2007)

Until recently, the active control of hearing sensitivity was not considered as an effective mechanism for the mitigation of the impacts of loud sounds in whales and dolphins. However, recently several studies have revealed the ability of whales and dolphins to actively control their hearing sensitivity. Originally this ability was demonstrated during echolocation. Measures of the auditory evoked potentials during echolocation have shown that whales and dolphins change their hearing sensitivity in order to optimize the perception of the echoes (Supin et al., 2005, 2010; Nachtigall and Supin, 2008; Linnenschmidt et al, 2012; Li et al, 2011; Supin and Nachtigall, 2012). The hearing sensitivity of a false killer whale *Pseudorca crassidens* was also shown to be more acute when the animal was searching by echolocation for targets than when targets were easily found (Supin et al, 2008). Later this capability was also demonstrated in conditions when it might serve to protect the hearing from the action of a loud sound. It has been found that a false killer whale and a bottlenose dolphin *Tursiops truncatus* are capable of dampening their hearing when a loud sound is preceded by a warning faint sound (Nachtigall and Supin, 2013, 2014). This in-advance damping of hearing had typical features of a conditioning effect and may therefore be an effective mechanism for hearing protection.

However, many features of the effect remained undefined after these studies and require further investigation. In particular, it is not known yet, whether the effect depends on the frequency of the loud sound, and whether it is frequency specific, i.e., whether the damping of sensitivity appears within overall frequency range of the subject's hearing or only within a certain frequency band. In the studies mentioned above, only one frequency of the loud sound was used, and the dampening of hearing sensitivity has been demonstrated also at only one (in the false killer whale) or two (in the bottlenose dolphin) frequencies. Therefore, to further understand the mechanisms and features of the conditioned hearing control in odontocetes, the spread of the conditioning dampening effect along the frequency scale, depending on the frequency of the loud, sound was investigated.

In order to reach this goal, the hearing sensitivity before the presentation of a loud sound was measured at various frequencies: below, at, and above the loud sound frequency. Hearing was measured using the auditory evoked potential (AEP) method since it allowed rapid audiometric measurements without preliminary training of the animal (Supin et al., 2001). Rhythmic trains of short pips were used as effective test stimuli yielding robust rhythmic AEP responses known as the envelope-following response (EFR) (Supin and Popov, 2007). This test was used for fast sensitivity measurements within a short time of warning before the loud sound.

Results

Evoked potential features in the baseline sessions.

In a baseline series, the hearing sensitivity was measured during three experimental sessions with presentation of only faint test stimuli, without loud sounds. The test stimuli were trains of short tone pips (*St* in Fig. 1). Each train contained 16 pips of a rate of 1000/s. The trains were repeated at a rate of 15/s during each measurement time; that time length randomly varying trial-by-trial from 5 to 30 s. Thus, 75 to 450 tone pips were presented within each trial. The test stimulus level was constant during each trial and varied randomly trial-by-trial from 85 to 120 dB re 1 μPa *rms* in order to determine the baseline hearing thresholds of the animal.

The stimuli produced well defined EFRs as exemplified in Figure 1A for a test frequency of 32 kHz. Each of the presented waveforms was obtained as a result of averaging of 1275 to 3500 original responses (test stimulus presentations) obtained during 10 trials, each of 75 to 450 stimulus presentations. The obtained waveforms featured a response to the pip train consisting of a series of waves of the same frequency (1 kHz) as the rate of tone pips within the test stimulus (1000/s). Data show a response lag as long as about 3 ms (beginning) to 5 ms (end) relative to the stimulus confirming the neurophysiological origin of the waveforms.

The EFR amplitude was dependent on the stimulus level. In the presented example shown in Fig 1, the response was maximal at levels of 115 to 120 dB re 1 μPa *rms* and the amplitude shows a decrease with stimulus level decrease and becomes indistinguishable from noise at a level of 90 dB re 1 μPa rms.

Evoked potential features in the conditioning sessions.

In each trial of the conditioning sessions during which both faint and loud sounds were presented, the faint test stimuli (the same pip trains as describe above) were repeated at the same rate of 15/s during a test time that randomly varied from 5 to 30 s (i.e., from 75 to 450 tone pip presentations during each trial). Immediately after the end of the test stimuli presentation (i.e., 5 to 30 s after starting the stimulation) a loud sound followed as a tone of a level of 165 dB re 1 µPa rms and duration of 5 s. Thus, the initial test stimuli served also as a warning signal that signaled that the loud sound was about to arrive. Therefore, for the conditioning sessions, these faint sounds preceding the loud one are referred to as test/warning stimuli, and the 5 to 30 s time of their presentation is referred to as test/warning time. The 5-to-30 s test/warning time was used because this rather short warning resulted in successful conditioned hearing dampening, whereas longer warning (15 to 75 s, mean 45 s) resulted in a less prominent effect (Nachtigall and Supin, 2014). Within the chosen limits of durations, trial-by-trial randomization of the test/warning time served to exclude the linking of a conditioning effect to a particular time after the warning signal onset.

The test/warning stimulus level was constant during each trial and varied randomly trial-by-trial from 85 to 120 dB re 1 µPa rms. During the test/warning time, brain responses were collected and averaged in the same manner as in the baseline sessions, to obtain an EFR to stimuli of various levels. The obtained EFR waveforms are exemplified in Figure 1B for the conditioning experiment with a test/warning signal of the 32 kHz carrier frequency (the same as in the baseline experiment shown in Fig. 1A). The loud sound following the test/warning signal was of 22.5 kHz frequency. Each of the waveforms was obtained by averaging of 1050 to 3500 (depending on the randomly varying test/warning times) of original waveforms recorded in 10 trials with test/warning stimulus level varied randomly from trial to trial.

In the same way as during the baseline trials, in the conditioning trials the EFR amplitude was also dependent on the stimulus level (Fig. 1B). However a comparison of Figures 1 A and B demonstrates that within all of the stimulus levels, the responses in the conditioning trials were substantially less than at the same levels found during the baseline trials. Unlike levels found during the baseline trials that resulted in very detectable responses at levels of 95 re 1 µPa rms and above, in the conditioning trials the response was absent at stimulus levels of 105 dB re 1 µPa and below.

Behavior associated with loud sound exposure

At the presentation of the first loud sound (after the completion of the baseline series), an element of aversive behavior of the subject was observed as a short backward movement, but the animal did not leave the listening position. This ‘aversive’ behavior disappeared during the first experimental (with loud sound exposures) session after five or six trials. During subsequent sessions, occasionally aversive behavior was observed as a little head shaking during the loud sound but the animal never left its position. No aversive behavior was observed during presentation of the faint test/warning sounds. During all of the sessions, the animal stayed in the position for measurement until it was called back by the trainer for appropriate fish reinforcement.

Evaluation of conditioning effects

In order to quantitatively evaluate hearing sensitivity, the frequency spectra of the averaged response waveforms were obtained. Each record was obtained by averaging of all original records in trials varied randomly from 5 to 30 s. The spectra were computed for a 16-ms long time window, from the 5th to the 21st ms after the beginning of the pip train, i.e., within a window of the same length as the stimulus pip train, with a 5-ms offset for the neurophysiological response lag. For the response waveforms exemplified in Fig. 1 A and B, the spectra are presented in Fig. 2 A and B, respectively. The spectra contained a definite peak at the frequency of 1 kHz which is equal to the stimulus pip rate. The magnitude of the 1-kHz spectrum component was used as a quantitative measure of the EFR magnitude and was plotted as a function of stimulus level.

These magnitude-vs-level functions were obtained for both baseline and conditioning phases of the study. The conditioning portion of the study was performed with all combinations of three frequencies of the test/warning signals, namely, 16, 22.5, and 32 kHz (i.e., 1/2 octave steps), and the same three frequencies of the loud sound. During each conditioning session, combinations of all three frequencies of the test/warning signals with one loud sound frequency were tested. Loud sounds of different frequencies were presented in different sessions. The magnitude-vs-test level functions were obtained by averaging the records from all of the experimental sessions (950 to 3600 original records averaged for each waveform and respective spectrum). Thus, overall, nine functions for the conditioning phase plus three baseline functions for the three test frequencies were obtained. All of them are presented in Fig. 3.

The results indicate that certain combinations of frequencies of the test/warning and loud sound resulted in substantial (5 to 15 dB) shifts of the magnitude-vs-level functions relative to the baseline for the same test frequencies, whereas other combinations resulted in no noticeable shift. In particular, at a 16-kHz test, the magnitude-vs-level function shifted relative the baseline by about 10 dB after the 16-kHz loud sound but not after other loud sounds (Fig. 3A). At a test frequency of 22.5 kHz, the shifts were about 10 dB after 16-kHz and 22.5-kHz loud sounds but not after the 32-kHz sound. Finally, at a test frequency of 32 kHz, the shifts were about 5 dB after 16-kHz and 32-kHz sound and about 15 dB after the 22.5-kHz sound. A statistical assessment (one-way ANOVA) of the effects of the loud sound provided results as follows: (1) For the test frequency of 16 kHz, the difference between the baseline and experimental (with the loud sound) data approached the standard criterion of statistical confidence for the loud sound frequency of 16 kHz ($p = 0.06$) whereas for the loud sound frequencies of 22.5 and 32 kHz the experimental data featured high probability of similarity with the baseline ($p = 0.96$ and $p =0.86$, respectively); (2) For the test frequency of 22.5 kHz, the experimental data significantly differed from the baseline at the loud sound frequencies of 16 and 22.5 kHz ($p = 0.03$ and $p = 0.03$, respectively) whereas the loud sound of 32 kHz resulted in high probability of similarity with the baseline ($p = 0.92$); and (3) finally, for the test frequency of 32 kHz, the experimental data significantly differed from the baseline at all frequencies of the loud sound: 16, 22.5, and 32 kHz ($p = 0.03$, $p = 0.005$, and $p = 0.01$, respectively).

To summarize all these data, response thresholds were evaluated for each of the conditioning combinations as well as for the baseline data. To evaluate a threshold, the oblique part of the magnitude-vs-level function was approximated by a straight regression line. The “oblique” part of the function was defined as a part where the gradient was not less than 10 nV *rms* per 5-dB increment, i.e., 2 nV/dB (see Methods). The intersection of the regression line with the zero-magnitude level was used as an estimate of the AEP threshold.

The resulting threshold estimates are presented in Figure 4A as threshold-vs-test frequency functions. The baseline function actually is a segment of the audiogram within the range of 16 to 32 kHz. The other functions show the modified audiogram segments as a result of conditioned sensitivity changes. The modifications were different depending on the frequency of the loud sound. This finding may be more obvious if the same data are presented as threshold shift dependence on the interrelation between the test/warning and loud sound frequencies (Fig. 4B). The threshold did not noticeably differ from the baseline when the test/warning sound frequency was 0.5 to 1 octave *below* the loud sound frequency (from –1 to –0.5 octaves in Fig. 4B). The thresholds did increase when the test/warning sound frequency was *equal to or above* the loud sound frequency (from 0 to +1 octave in Fig. 4B). The maximum threshold increase was observed at a test/warning frequency 0.5 oct above the loud sound frequency (15.5 dB at 22.5 kHz loud sound and 32 kHz test/warning signal; 10.0 dB at 16 kHz loud sound and 22.5 kHz test/warning signal).

Discussion

Conditioned or non-conditioned effect?

The study was designed to investigate features of the effects of hearing conditioning expected when the appearance of a loud sound can be predicted by a preceding faint sound. However, in the experiments described above, trials with the loud sound followed one another many times during each experimental session. Therefore, direct (non-conditioned) effects of the loud sound such as temporary threshold shift (TTS) could not be totally excluded by definition. The question concerning the nature of the dampening effect seems even more important because of the data presented herein on frequency specificity of this effect. Indeed, TTS is more prominent at frequencies equal to or higher than the fatiguing-sound frequency (Schlundt et al., 2000; Nachtigall et al., 2004; Finneran et al., 2007; Lucke et al., 2009; Mooney et al., 2009; Popov et al., 2011, 2013). Thus, both any non-conditioned TTS effect and the effect described in the present study feature some similar frequency specificity.

Arguments in favor of the conditioned, rather than non-conditioned, nature of the observed hearing dampening were presented when the effect was previously described in a false killer whale (Nachtigall and Supin, 2013). Moreover, recently published data obtained in the same subject and under the same experimental conditions as in the present study have shown that the dependence of the effect on temporal interrelations between the test/warning and loud sound indicates the conditioning nature of the observed dampening of hearing sensitivity (Nachtigall and Supin, 2014). The dampening effect did not depend on inter-trial intervals, i.e., on how frequently loud sounds were presented and how long the delay was between the loud sound and the subsequent test. On the contrary, the effect depended on the duration of the warning before the loud sound. Both these features of the hearing dampening effect are contradictory to the predictions of any unconditioned nature of the dampening effect and are not contradictory to the conditioned nature of the effect because characteristics of the conditioning stimuli may influence the success of conditioning.

The absence of unconditioned hearing dampening (TTS) within the present experiment is reasonable because of the rather low sound exposure level (SEL) of the loud sounds. The loud sound of SPL of 165 dB re 1 μPa and duration of 5 s has SEL as low as 172 dB re 1 μPa^{2}s. It is much lower than the SEL producing TTS in the majority of odontocete studies (Finneran et al., 2000, 2007; Nachtigall et al., 2003; Popov et al., 2011, 2013, 2014). The total exposure during a whole session (54 trials, see “Methods”) was 189 dB re 1 μPa^{2}s, however, this total exposure consisted of short (5 s) exposures separated by long (50 s or longer) intervals (i.e., duty cycles less than 0.1). This sort of intermittent exposure is much less effective than continuous exposure at producing effects like TTS (Finneran et al., 2010) due to partial recovery during the long time periods between exposures. TTS recovery during just a few minutes after low-SEL exposures has been demonstrated directly (Popov et al., 2013, 2014). So the absence of long-term TTS is very reasonable, and the hearing dampening effect investigated in the present study may be assumed therefore to be a manifestation of the conditioning-based control of hearing sensitivity.

Assuming that the observed effect of dampening hearing sensitivity was conditioned, these data indicate similar frequency dependence between the conditioning effect and TTS. It is possible that this similarity might indicate the involvement of common, or similar, mechanisms of these two processes.

Generalization of the data

The generality and implications of these data are limited by the fact that they were obtained from only one subject. However the effect obtained in the present study was similar to that described previously in another odontocete subject of another species, the false killer whale (Nachtigall and Supin, 2013). Despite some quantitative differences that may result from a difference in the subject species, individual animals, and signal parameters, qualitatively the dampening of hearing when a loud sound is preceded by a faint warning sound looked similar in both investigations. This would argue in favor of the hypothesis that the conditioned control of hearing sensitivity is a feature of the odontocete auditory system.

Frequency specificity and potential mechanisms of the hearing conditioning effect

A new finding of this study was that the hearing dampening did not cover the whole frequency range of hearing, or a certain constant part of the hearing range, but appeared within a limited frequency band linked to the loud sound frequency. The dampening always appeared at frequencies that were equal to or above the loud sound frequency. This finding is relevant to understanding the mechanisms of the hearing conditioning effect.

Among the possible mechanisms of hearing sensitivity control, first of all, the acoustic, or stapedial, reflex is considered because of many indications of hearing regulation in this way, i.e., by reflexively tightening the stapedial muscle in the middle ear. In humans, this mechanism is responsible for the reduction of hearing sensitivity produced by loud sounds (Hung and Dallos, 1972). Bats, during echolocation, contract their middle ear muscles synchronously with vocalization to attenuate the amount of self-stimulation by as much as 20 dB (Henson, 1965). So the role of the same mechanism in conditioned hearing control in odontocetes might be hypothesized. However, the frequency specificity found within this study is contradictory to this explanation. Execution of the sensitivity control through one and the same stapedial muscle cannot selectively influence different frequency bands depending on frequency of the reflex-triggering signal.

More probably, the mechanism will be found in neuronal events. The auditory system includes a variety of descending pathways that control the auditory perception at several levels, including the cochlear level (Winer, 2005). It was shown long ago that activation of the descending pathways suppresses the cochlear responses (Galambos, 1956; Suga and Schlegel, 1972). This control can be carried out, in particular, through the outer hair cells which form the "active mechanism" responsible for high sensitivity and acute frequency tuning of hearing. Descending auditory pathways project directly to the outer hair cells, thus being capable of controlling hearing sensitivity (Guinan, 2006). Apart from the control through the outer hair cells, descending regulatory influences between the auditory centers are also possible. Contrary to the contraction of the stapedial muscle, all the neural influences may be addressed to particular loci of the tonotopic projections within the auditory system, thus being frequency specific. Involvement of this mechanism in the conditioned hearing control in odontocetes cannot be excluded, although direct indications are absent at present.

Methods

Experimental facilities and subject

The study was carried out in the facilities of the Hawaii Institute of Marine Biology, Marine Mammal Research Program. The subject was a captive born female bottlenose dolphin known to be 28 years old with a long history of experimental work (e.g. Harley et al, 1996). The subject was trained to accept suction-cup electrodes for brain-potential recording, to swim into a hoop station and to listen to the sound stimuli. She had a moderate hearing loss that involved a high frequency cut-off at 45 kHz and increased thresholds below this cut-off; her hearing thresholds within a range from 16 to 38 kHz were 80 to 90 dB re 1 μPa which was higher than typical of bottlenose dolphins recently wild-caught (Popov et al., 2007) and higher than in-captivity held bottlenose dolphins and in many other odontocete species (rev. Au, 1993; Supin et al., 2001; Au and Hastings, 2008), however it was considered as still suitable for investigation of basic hearing processes. The subject was housed in a floating pen complex. Experiments were carried out in a section of the pen complex 8 × 10 m in size.

Experimental procedure

Each experimental session started by calling the subject to the trainer and attaching surface latex suction cups containing electrodes for brain-potential recording. The 10-m long thin flexible cables connecting the suction cups to the equipment allowed the dolphin to move over much of the experimental pen. After attaching the suction cups, 54 experimental trials were run during a daily session.

Each trial started by sending the subject to a listening station. The station was a hoop fastened at a depth of 80 cm below the water surface. During stationing, low-level test sounds were played which served to measure hearing sensitivity (see below: "Signal parameters and presentation timing"). During the presentation of the test sounds, brain potentials, specifically EFR evoked by the test stimuli, were recorded. These responses served to measure hearing sensitivity (see below: "AEP acquisition and hearing-sensitivity assessment"). In baseline measurement trials, only these test sounds were presented. In experimental (conditioning) trials, immediately after the test sound, a high-level (loud) sound was played. Since the test sounds always preceded the loud sound, they also served as conditioning stimuli warning the subject of the oncoming loud sound. At the completion of each trial, a secondary reinforcing whistle was blown and the subject was called back from the listening station and received fish reinforcement.

Signal parameters and presentation timing

The duration of the test/warning sound in which the hearing was measured varied randomly trial-by-trial from 5 to 30 s so that the animal could not anticipate the end of the warning and become conditioned to the time. The loud sound was always 5-s long, played immediately after the test/warning time. Trials followed one another with inter-trial intervals of 55 ± 5 s.

The test/warning signals were rhythmic trains of tone pips, each train 16-ms long containing 16 pips at a rate of 1 kHz. The trains were played at a rate of 15/s during the test/warning time as specified above (Fig. 5A). Each pip contained 8 cycles of a carrier frequency (Fig. 5B). The carrier frequency was 16, 22.5, or 32 kHz. From trial to trial, levels of the test/warning signals varied up and down from 85 to 120 dB re 1 μPa *rms*, by 5-dB steps, i.e., total of 8 levels. Variation of the test signal level was random and was presented as a method of constants rather than a staircase procedure, i.e., the level did not depend on the response presence or absence. Independent of the response presence or absence, all the 85 to 120 dB range was examined to obtain information on the response magnitude at both threshold and supra-threshold levels.

In every session, the test/warning signals were presented at all three frequencies (16, 22.5, and 32 kHz), 6 of 8 levels of each frequency. Each combination of the test/warning signal frequency and level was repeating 3 times, so each session contained overall of 54 trials.

The loud sound was a tone of a sound pressure level (SPL) of 165 dB re 1 μPa *rms* lasting 5 s. Loud sound frequencies were 16, 22.5, or 32 kHz.

Instrumentation for sound generation and data collection

Both the test/warning and loud sounds were digitally synthesized by a standard personal computer using a custom-made program (virtual instrument) designed with the use of LabVIEW software (National Instruments, Austin Texas, USA). The synthesized signal waveforms were played at an update rate of 256 kHz through a 16-bit digital-to-analog converter of a USB-6251 acquisition board (National Instruments, Austin Texas, USA). The test signals were amplified by a custom-made power amplifier (passband of 1 to 150 kHz), attenuated by a custom-made low-noise resistor attenuator, and played through an ITC-1032 piezoceramic transducer (International Transducer Corporation, Santa Barbara, California, USA) positioned at a depth of 80 cm (i.e., the same depth as the hoop station center) at a distance of 1 m in front of the animal's head.

Signals for the loud sound were amplified by a Hafler P3000 power amplifier (Hafler, Tempe, Arizona, USA) and played through the same transducer. The transducer was connected alternatively either to the test sound attenuator or to the loud sound power amplifier through an electromagnetic relay, so the background noise of the power amplifier output never overlapped the low-voltage (down to a few mV) test signals. The reconnection was done simultaneously with the loud sound onset, to avoid any cue preceding the loud sound. Both test and loud sounds were calibrated by a B&K 8103 hydrophone (Bruel & Kjaer, Naerum, Denmark) positioned in the hoop station in the absence of the subject.

Brain potentials were picked up through 10-mm gold-plated surface electrodes mounted within 50-mm latex suction cups, the active electrode at the vertex, and reference electrode at the dorsal fin. Brain potentials were fed through shielded cables to a balanced custom-made brain-potential amplifier based on an AD620 chip (Analog Devices, Norwood MA, USA) and amplified by 60 dB within a frequency range from 0.2 to 5 kHz. The amplified signal was entered into a 16-bit analog-to-digital converter which was one A/D channel of the same NI USB-6251 acquisition board that served for sound generation. The digitized signals were processed in a standard personal computer.

Brain potential acquisition and hearing sensitivity assessment

The hearing sensitivity assessment was based on recording the EFR to the test tone pips. The brain potentials were averaged on-line within every trial. EFR records obtained by on-line averaging were sorted according to the stimulus frequency and level and were additionally averaged off-line among the trials to obtain final low-noise EFR records. A 16-ms long part of the record, from 5th to 21st ms, containing the EFR was Fourier transformed to obtain its frequency spectrum. The spectrum peak magnitude at the stimulation rate (1 kHz) was taken as the EFR magnitude. The EFR magnitudes evaluated in this way were plotted as a function of test-signal level. An oblique part of the function was approximated by a straight regression line (see Fig. 3 above). This “oblique” part of the function was defined as a part with point-to-point gradients not less than 10 nV per 5-dB level increment (2 nV/dB). This arbitrary criterion was chosen as allowing to separate the level-dependent segment of the voltage-vs-level function from its flat parts presenting the background noise at subthreshold stimulus levels and “saturation” range at high stimulus levels. The point of interception of the regression line with the zero response magnitude level was taken as the threshold estimate (Supin and Popov, 2007).

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Author contribution

The authors contributed equally to the completion of this effort.

Competing interests

No competing interests declared.

List of Abbreviations

AEP – Auditory evoked potential
EFR – envelope following response
Oct – octave
PTS – permanent threshold shift
RMS – root mean square
SEL – sound exposure level
SPL – sound pressure level

TTS – temporary threshold shift

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Figure captions

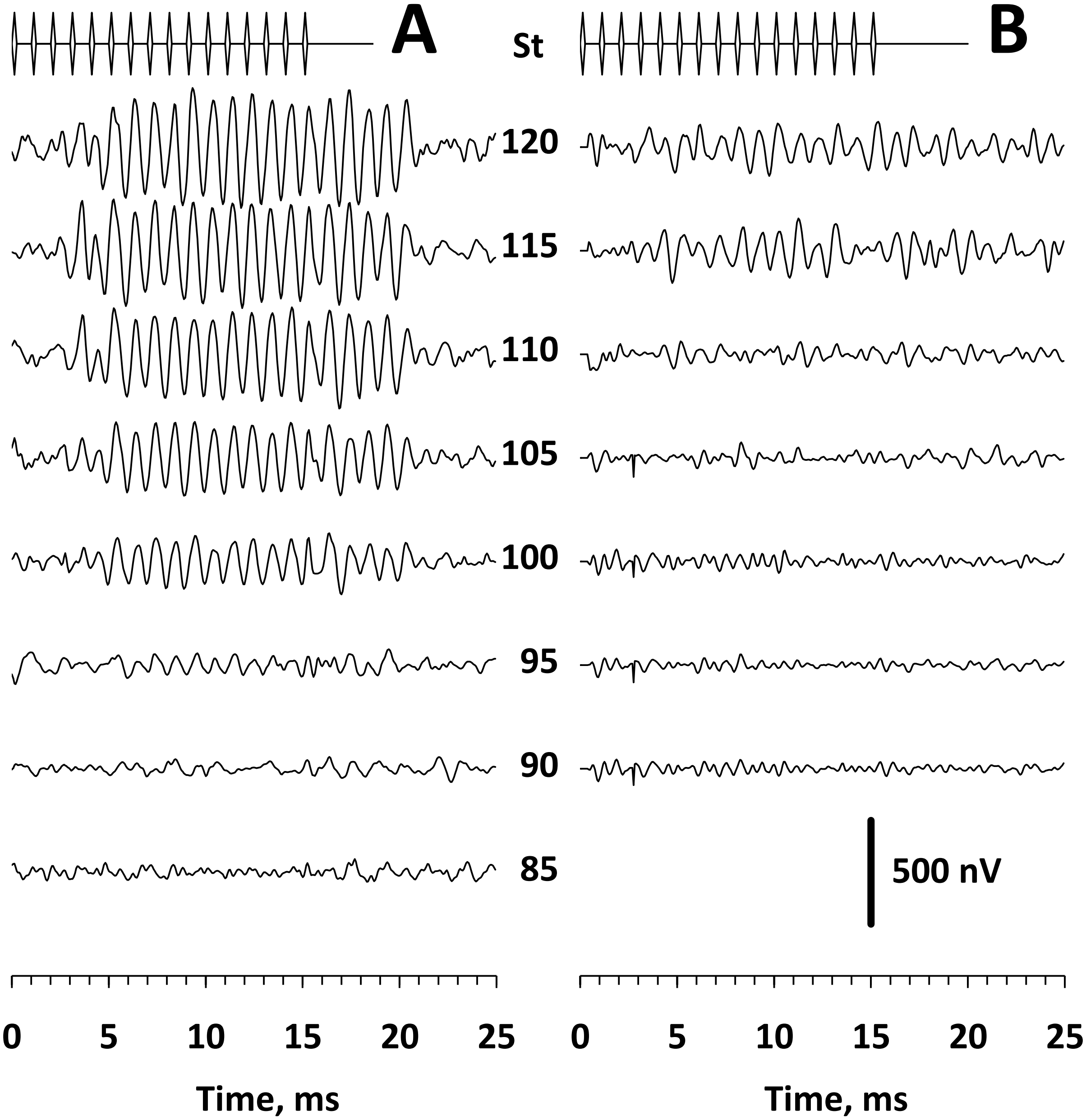
Fig. 1. Example of EFR records at various test-stimulus levels in baseline (no loud sound) measurements (A) and conditioning (with the loud sound of 22.5 kHz) experiments (B). Test frequency 32 kHz. Test-stimulus levels are indicated near the records in dB rms re 1 μPa, *St* – stimulus (pip train) envelope. In both (A) and (B), each record was obtained by averaging of all original records in trials varied randomly from 5 to 30 s.

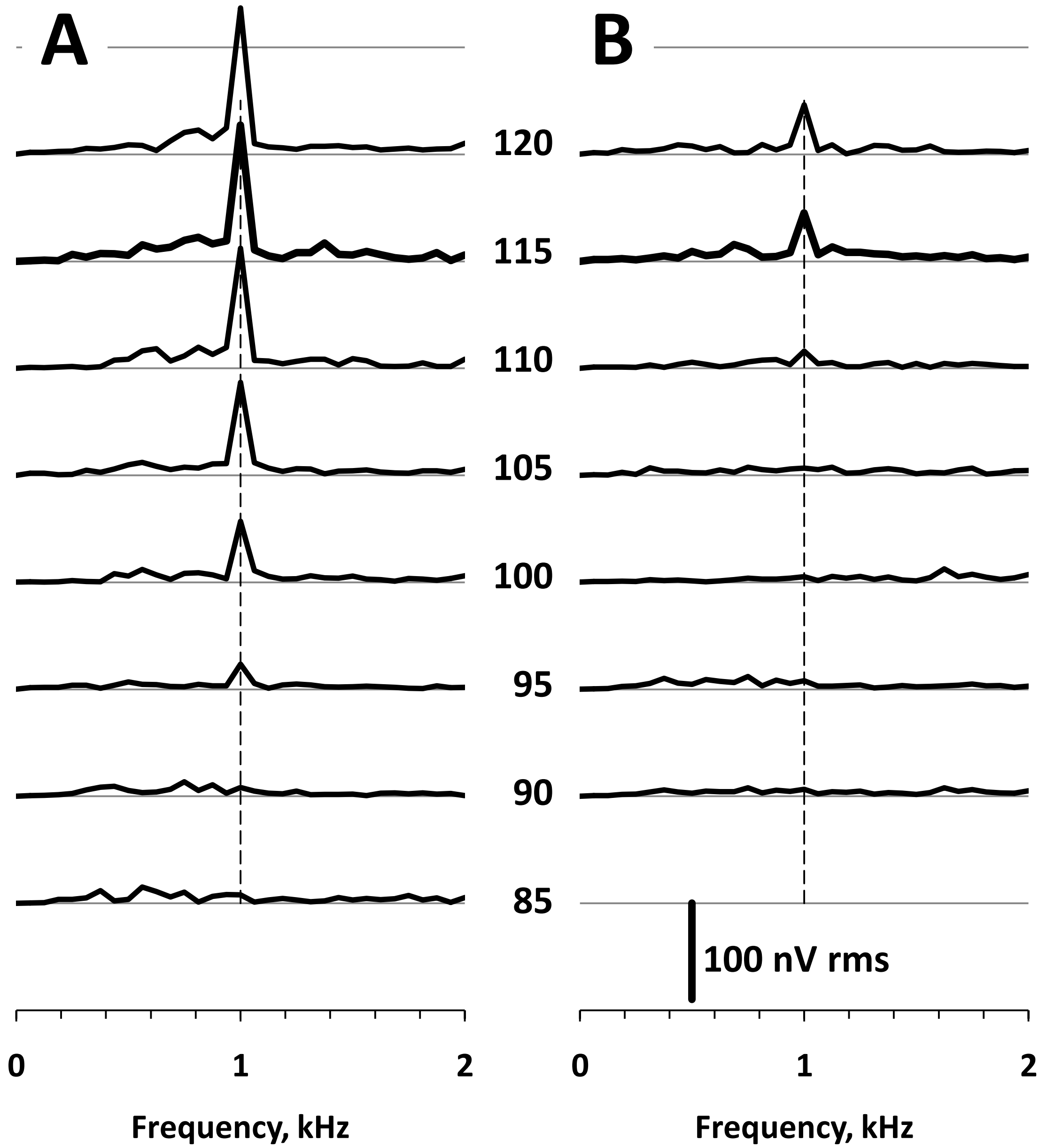
Fig. 2. Frequency spectra of waveforms presented in Fig. 1 (A and B, respectively). Test-stimulus levels are indicated near the records in dB rms re 1 μPa. Vertical dashed lines mark the spectrum peak at the response frequency (1 kHz).

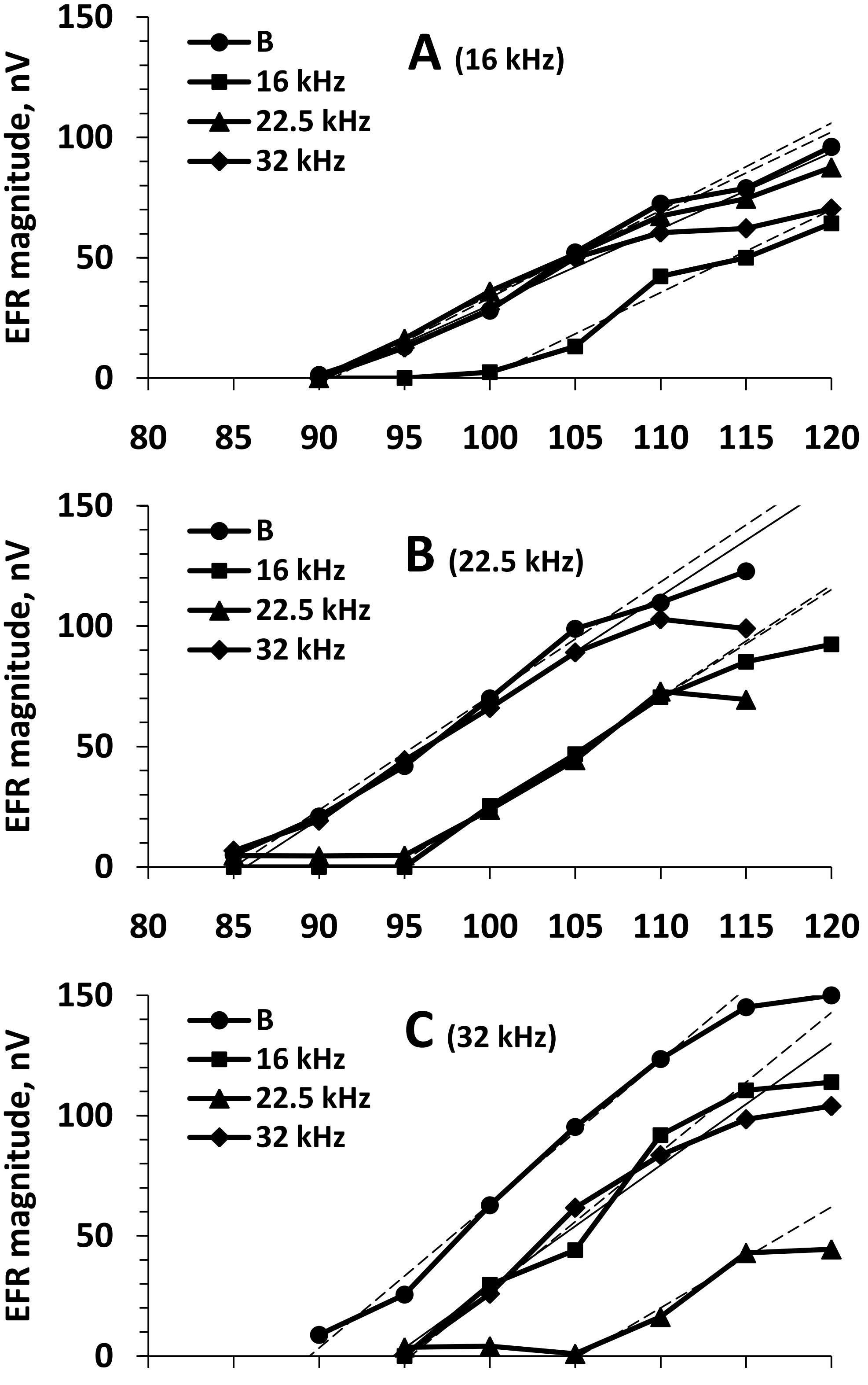
Fig. 3. EFR magnitude dependence on test signal level at test frequencies of 16 kHz (A), 22.5 kHz (B), and 32 kHz (C) in baseline experiments (B) and in experiments with loud sound after each test/warning signal; frequencies of the loud sounds (16, 22.5, and 32 kHz) are indicated in the legends. Dashed straight lines – regression lines approximating the oblique segments of the functions.

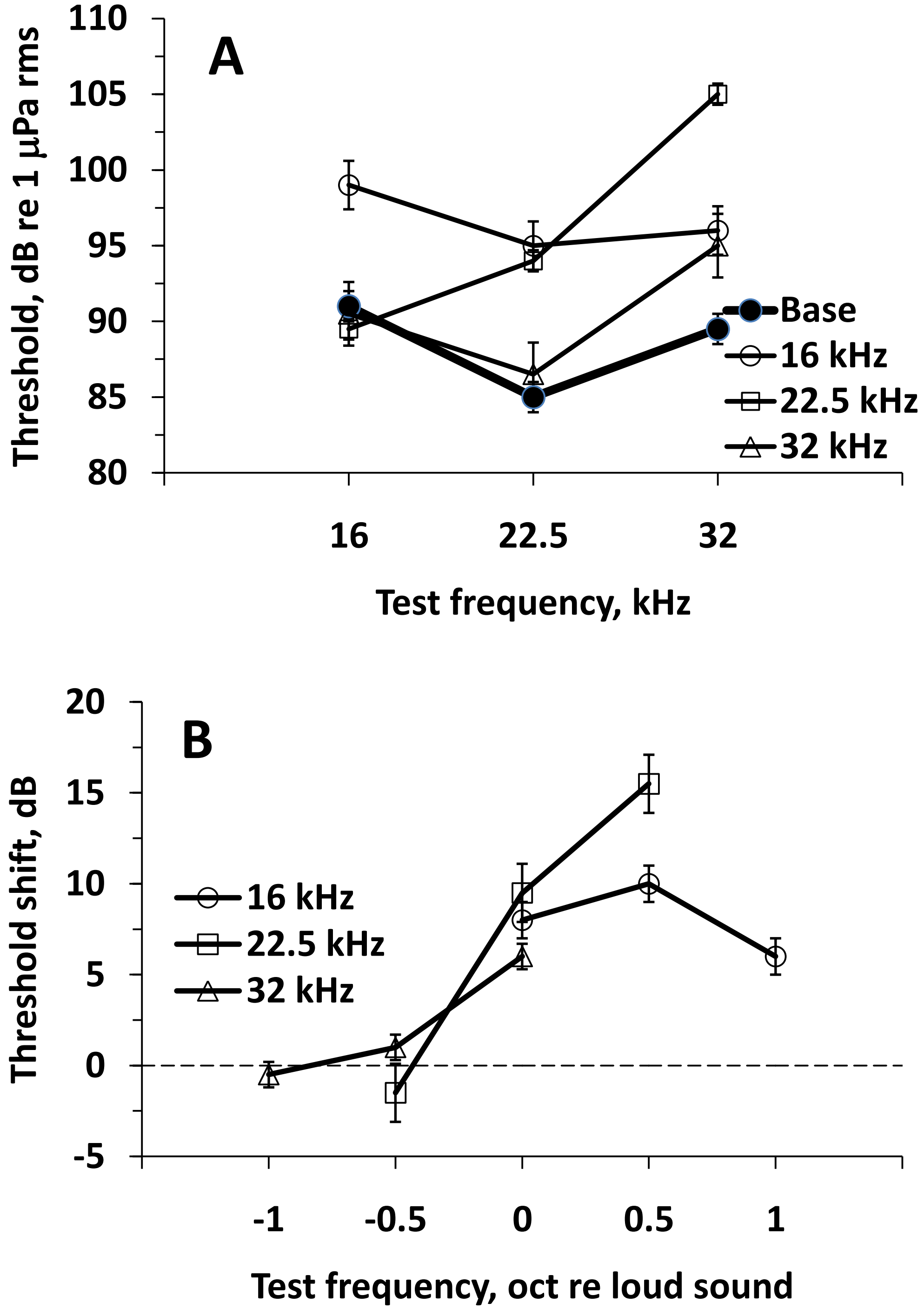
Fig. 4. Thresholds and conditioned threshold shifts as functions of test frequency. **A**. Thresholds. Test frequency is specified in kHz and threshold in dB re 1 μPa, “Base” – baseline thresholds; “16”, “22.5”, and “32” - thresholds in experiments with loud sound of the specified frequency after each test/warning signal. **B**. Threshold shifts. Test frequency is specified in oct relative the frequency of the loud sound, and threshold shifts are specified in dB re baseline taken as zero (dashed line); “16”, “22.5”, and “32” – threshold shifts in experiments with loud sound of the specified frequency after each test/warning signal. Error bars – standard errors for regression line crossing zero level.

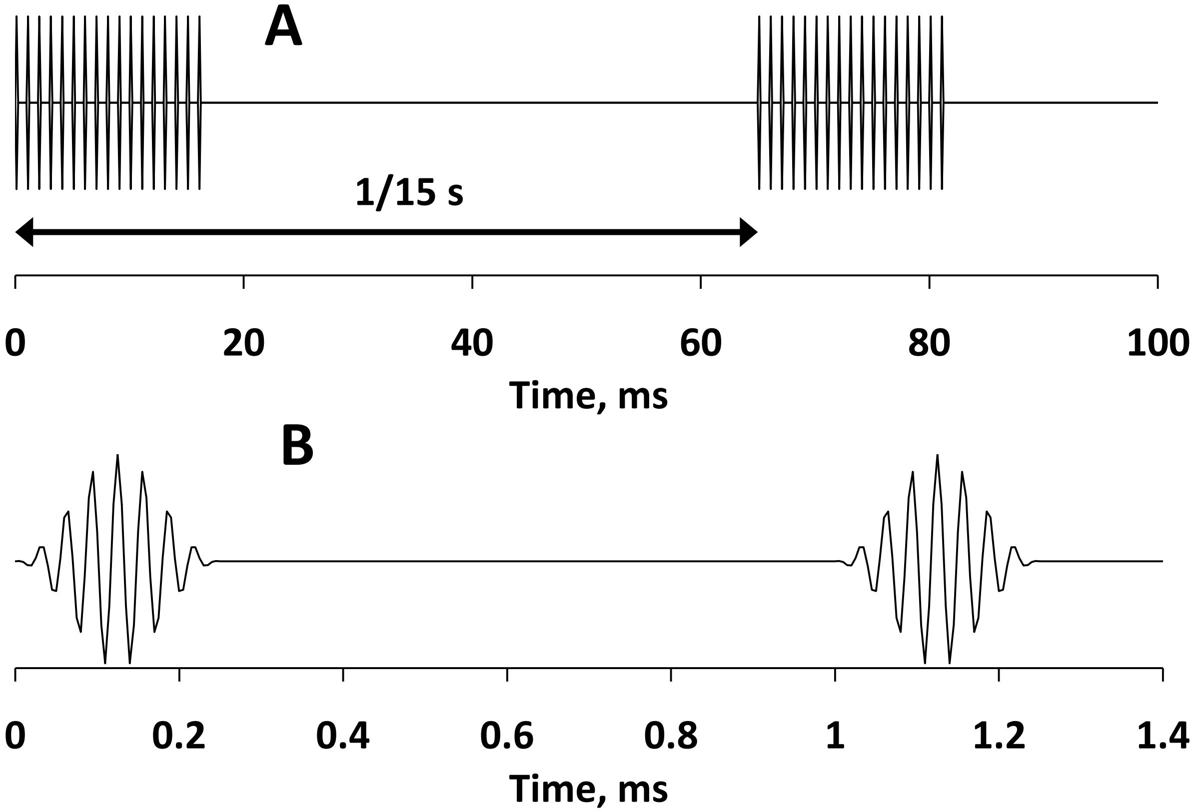
Fig. 5. Waveforms of test/warning stimuli at different time scales. **A**. Compressed time scale, two successive pip trains are presented. **B**. Extended time scale, two successive pip trains of 16 pips in the train are presented.


A
St
B
120
115
110
105
100
95
90
85
500 nV
0
5
10
15
20
25
Time, ms
0
5
10
15
20
25
Time, ms


A
B
120
115
110
105
100
95
90
85
100 nV rms
0
1
2
0
1
2
Frequency, kHz
Frequency, kHz


EFR magnitude, nV
A (16 kHz)
B (22.5 kHz)
C (32 kHz)
B
16 kHz
22.5 kHz
32 kHz
0
50
100
150
80
85
90
95
100
105
110
115
120
Test level, dB re 1 μPa


A
Threshold, dB re 1 µPa rms
110
105
100
95
90
85
80
16
22.5
32
Test frequency, kHz
Base
16 kHz
22.5 kHz
32 kHz
B
Threshold shift, dB
20
15
10
5
0
-5
-1
-0.5
0
0.5
1
Test frequency, oct re loud sound
16 kHz
22.5 kHz
32 kHz


A
1/15 s
0
20
40
60
80
100
Time, ms
B
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Time, ms