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RESEARCH ARTICLE

Expectancy and conditioned hearing levels in the bottlenose dolphin (*Tursiops truncatus*)

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ABSTRACT

The hearing sensitivity of a bottlenose dolphin for a warning sound, when the exact time of the arrival of a loud sound could or could not be predicted, was measured. Sensitivity was measured when the time of onset of the loud sound was randomly varied (random-variation sessions) and when the time of onset of the loud sound and the pattern of stimulus levels was constant (fixed-stimulus sessions). The loud sound was kept the same in both of the series. The mean duration and mean range of the levels of the test/warning signal were also kept equal across experimental sessions. Hearing sensitivity was measured using the auditory evoked potential method with rhythmic trains of short pips as test stimuli. With randomly varied warning sounds, thresholds before the loud sound were on average 10.6 dB higher than the baseline thresholds. With fixed warning signals, thresholds were on average 4.4 dB higher than the baseline thresholds. Considering that the loud sounds were identical, the difference between the random-variation and the fixed-stimulus sessions cannot be explained by a direct (unconditioned) influence of sound exposure. Therefore, the data provide reliable evidence for the conditioning nature of the hearing-dampening effect and also demonstrate that hearing sensitivity change also depends on when the animal can expect the loud sound to occur.

KEY WORDS: Echolocation, Hearing, Conditioning, Sensitivity control, Auditory evoked potential, Odontocete

INTRODUCTION

Since the stranding of beaked whales and other species of whales associated with Navy sonars (Evans and England, 2001), there has been an effort to understand and mitigate the effects of intense underwater sound on marine mammals. Echolocating whales, including dolphins, have particularly sensitive high-frequency hearing (Nachtigall et al., 2000; Southall et al., 2007). Given that some whales produce echolocation signals exceeding 235 dB re. 1 μPa under water (Møhl et al., 2003) and that their ears are located relatively close to their sound production structures (Cranford et al., 1996; Wartzok and Ketten, 1999; Surlykke et al., 2014), an exploration of the functional mechanisms evolved to protect sensitive hearing and therefore mitigate the effects of loud sound seems reasonable. These mechanisms were found, in particular, as a component of the sonar in whales and dolphins. An examination of auditory evoked potentials (AEPs) while a false killer whale echolocated, found that the whale substantially changed hearing sensitivity depending on the echolocation target situation (Supin et al., 2008). The whale maximized its hearing sensitivity to hear a quiet echo and reduced its sensitivity at high echo levels.

Given that ability to change hearing sensitivity, it also seemed reasonable to test whether the false killer whale, the bottlenose dolphin (Nachtigall and Supin, 2013, 2014, 2015) and the beluga whale (Nachtigall et al., 2016) would likewise dampen hearing when signaled that a loud sound was about to arrive. All of these odontocetes changed their hearing sensitivity when a warning signal preceded the arrival of a relatively loud sound, thereby mitigating its effects.

When attempting to measure whether an animal changes its hearing when signaled loud sounds are presented, ideally one would measure how sensitive the animal's auditory system is during the actual presentation of the loud sound. But it is impossible to test hearing sensitivity (i.e. the ability to detect faint sounds) in the background of loud sounds. Therefore, a technique was developed to measure hearing during the time an animal might expect to hear a loud sound (Nachtigall and Supin, 2013). That procedure involved randomly varying the duration of the warning signal that indicated that the loud sound was about to arrive. Hearing thresholds were measured using the warning sounds as test stimuli. The animal could not know exactly when to expect the loud sound to start; therefore, the animal was expected to dampen its hearing sensitivity as soon as the warning sound was turned on. This dampening, if it were to occur, could be detected before the loud sound. Using this approach, the dampening of hearing sensitivity prior to the loud sound was in fact observed. Generally, a false killer whale (Nachtigall and Supin, 2013), a bottlenose dolphin (Nachtigall and Supin, 2014, 2015) and a beluga whale (Nachtigall et al., 2016) changed their hearing thresholds by around 15 dB in anticipation of the loud sound. This effect was considered as a sort of conditioning when the warning signal played the role of a conditioning stimulus and protection from the loud sound was the reinforcement.

Suppose, however, the time prior to the presentation of the loud sound had not been varied. If the warning sounds had always been of the same duration, an animal could well have used the signal to change hearing sensitivity only during the loud sound or immediately before the loud sound onset, but not necessarily in advance during the warning sound. In this case, any changes in hearing sensitivity would not have been evident and would not have been observed.

This reasoning prompted an additional look at hearing sensitivity in a dolphin in two different conditions: (1) when warning signals prior to the loud sound varied randomly in duration and level, and (2) when warning signals were of constant duration and level. It was expected that the decrease in hearing sensitivity prior to the loud sound would be more pronounced in conditions of random variation than in conditions of constant warning signals. This result was expected to reveal the animal's ability to control and to time hearing sensitivity according to the expected temporal pattern of the auditory scene. Much previous work (e.g. Nachtigall and Supin,

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2013, 2014, 2015) assumed that sensitive measures of hearing change required a random variation of the warning signal. This effort provided a test of that assumption.

A comparison of hearing sensitivity in the two conditions might also answer a question as to whether the changes in hearing sensitivity might be due to the direct unconditioned effects of the loud sound itself in a manner similar to what occurs during a temporary threshold shift (TTS). If an equal amount of hearing sensitivity change occurred independent of condition, one might suspect an effect like TTS as the cause. Alternatively, if hearing were to change differently depending on randomly varying or constant warning signals despite equal loud sounds, it could not be explained by direct non-conditioned action of the loud sound. This would provide additional support for the hypothesis of the conditioned nature of the hearing sensation level change.

This experiment was therefore designed to answer two questions by comparing hearing sensation change prior to a loud sound with a randomly varied warning sound versus a fixed warning sound: (1) can the dolphin learn to time its hearing sensation change; and (2) can the dolphin demonstrate a different amount of hearing change when arrival of the loud sound is predictable as compared to trials in which the onset of the loud sound is not predictable?

MATERIALS AND 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 laboratory-born female bottlenose dolphin [*Tursiops truncatus* (Montagu 1821)], BJ, known to be 29 years old with a long history of experimental work. The work was approved by the Institutional Animal Care and Utilization Committee of the University of Hawaii. 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 the 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 bottlenose dolphins and other odontocete species (Au and Hastings, 2008), which were generally between 50 and 70 dB. However, her hearing was considered as still suitable for this investigation of basic hearing change processes (Nachtigall and Supin, 2015). The subject was housed in a floating pen complex off of Coconut Island in Kaneohe Bay. Experiments were carried out in a section of the floating pen complex that was 8×10 m wide and 6 m deep.

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 electrodes to the equipment allowed the dolphin to move over much of the experimental pen. After the suction cups had been attached, 36 experimental trials were run during each 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 (in baseline sessions, see below) or test/warning (in conditioning sessions, see below) sounds were played, which served to measure hearing. During the presentation of the test or test/warning sounds, brain potentials evoked by the test stimuli were recorded. These responses served to measure hearing sensitivity. Two kinds of sessions were conducted: baseline and conditioning.

During the baseline sessions, only low-level sounds were presented; below, they are referred to as 'test sounds'. During conditioning sessions, immediately after the low-level sound, a high-level (loud) sound was played. In these sessions, the low-level sounds served both to measure hearing and as conditioning warning sounds, so hereafter the low-level sounds are referred to as test/warning sounds in the conditioning sessions. 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 for sitting and listening. In all sessions (baseline and conditioning), trials followed one another with inter-trial intervals of 55±5 s.

In order to ensure proper comparisons to conditioning sessions (see below), two sorts of baseline sessions were conducted, with either randomly varying or fixed test signals.

In sessions with randomly varying signals (below referred to as random-variation sessions), test signals randomly varied trial-by-trial in both duration and level. The duration varied in six 5 s steps from 5 to 30 s, with a mean of 17.5 s. The test signal level was constant during each trial and varied randomly trial-by-trial in six 5 dB steps from 90 to 115 dB re. 1 µPa rms (root mean square), with a mean of 102.5 dB re. 1 µPa rms (Fig. 1A). This manner of variation of the stimulus parameters was used to make the animal incapable of knowing what to expect about the level or how long the trial would be. Each combination of a stimulus duration and level was presented once a session, for the total of 36 trials.

In sessions with fixed stimuli, the test stimuli were identical in all trials (below referred to as fixed-stimulus sessions; Fig. 1B); each trial had all of the levels and one fixed duration of 17.4 s. Each trial started at a stimulus level of 90 dB re. 1 µPa rms for 2.9 s, followed immediately by 2.9 s of a stimulus presented at 95 dB re. 1 µPa rms followed by others in 5 dB increments up to the level of 115 dB re. 1 µPa rms. There were a total of six levels with a mean of 102.5 dB re. 1 µPa rms. Given these were all the same, the animal could know what to expect. Similar to the random-variation sessions, each fixed-stimulus session contained 36 trials.

During the conditioning sessions, both random-variation and fixed-stimulus sessions were presented. The ranges and manners of variation of stimulus duration and level were the same as in the

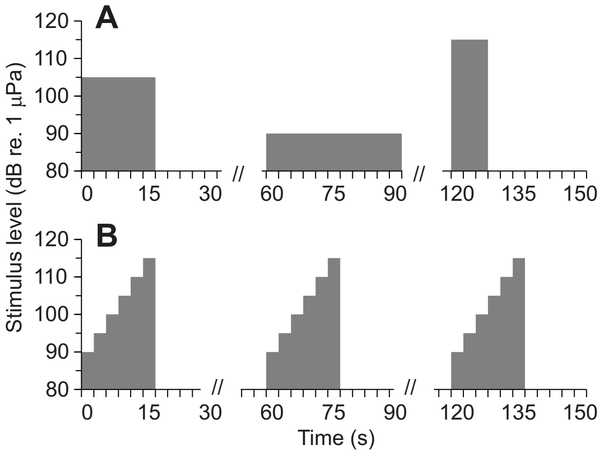

Fig. 1. Temporal diagrams of test stimulus presentation in baseline sessions. (A) Random variation. (B) Fixed stimuli. In each diagram, three arbitrarily chosen successive trials are presented. Bars represent levels of the test signals.

baseline sessions. Each conditioning session, either random variation or fixed stimulus, contained 36 trials. The difference from the baseline sessions was that each of the test/warning sounds was followed by a loud sound (Fig. 2).

Signal parameters

The test or test/warning signals were identical in both baseline sessions (test signals) and conditioning sessions (test/warning signals). They were rhythmic trains of tone pips, with each 16 ms-long train containing 16 pips at a rate of 1 kHz to maximize signal visibility (Supin and Nachtigall, 2013). The trains were played at a rate of 15 s^{-1} during the test or test/warning time (Fig. 3A). Each pip contained 8 cycles of a carrier frequency of 32 kHz enveloped by a cosine function (Fig. 3B). Levels and durations of the test or test/warning signals varied differently in random-variation and fixed-stimulus sessions, as described in ‘Experimental procedure’, above.

Brain potential acquisition and hearing sensitivity assessment

The hearing sensitivity assessment was based on recording the rate-following response (RFR), which is a kind of AEP evoked by rhythmic stimuli (tone pip trains). For this, 25 ms-long epochs synchronous with the pip-train stimuli were extracted from the brain potentials and were averaged on-line within every trial. Recording and sound presentation began simultaneously but brain response records did not reveal following for 4–5 ms. 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 a final low-noise RFR record. For the off-line average, each on-line record was weighted proportional to the number of on-line averaged epochs. A 16 ms-long part of the final off-line average record, from the 5th to the 21st millisecond, containing the RFR, was Fourier transformed to obtain its frequency spectrum. The spectrum peak magnitude at the stimulation rate (1 kHz) was taken as the RFR magnitude. The RFR magnitudes evaluated in this way were plotted as a function of signal level. An oblique part of the function was approximated by a straight regression line. This oblique part of the function was defined as a part with point-to-point gradients not less than 10 nV per 5 dB increment (2 nV dB^{-1}). This arbitrary criterion was chosen as allowing separation of the level-dependent segment of the voltage versus level function from its flat part representing the background noise. The point of interception of the regression line with the zero response magnitude level was taken as the threshold estimate (Supin and Popov, 2007).

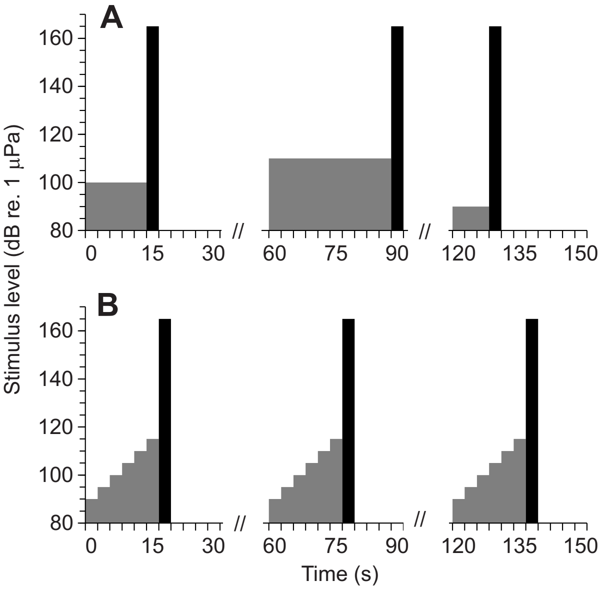

Fig. 2. Temporal diagrams of test stimulus presentation in conditioning sessions. (A) Random variation. (B) Fixed stimuli. In each diagram, three arbitrarily chosen successive trials are presented. Gray bars, levels of the test/warning signals; black bars, levels of the loud sound.

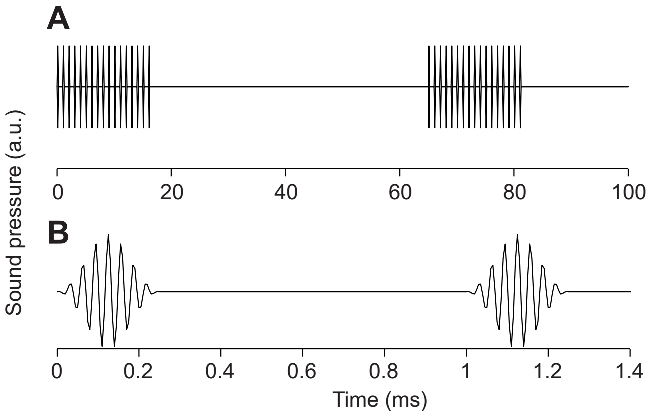

Fig. 3. Waveforms of test/warning stimuli at different time scales. (A) Compressed time scale; two successive pip trains are presented. (B) Extended time scale; two successive pips of the 16 pips in the train are presented.

Instrumentation for sound generation and data collection

All sound signals (test, 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, TX, USA). The synthesized signal waveforms were played at an update rate of 512 kHz through a 16-bit digital-to-analog converter of a USB-6251 acquisition board (National Instruments).

The test or test/warning sounds were amplified by a custom-made power amplifier (passband of 1–150 kHz), attenuated by a custom-made low-noise resistor attenuator, and played through an ITC-1032 piezoceramic transducer (International Transducer Corporation, Santa Barbara, CA, 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, AZ, USA) and played through the same transducer. The transducer was connected alternatively either to the test/warning sound attenuator or to the loud sound power amplifier through an electromagnetic relay, so the background noise of the Hafler P3000 output never overlapped the low-voltage (down to a few mV) test/warning signals. The reconnection was done simultaneously with the end of the test/warning sound and onset of the loud sound, to avoid any cue preceding the loud sound. Both test (test/warning) 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 diameter gold-plated surface electrodes mounted within 50 mm latex suction cups, with the active electrode at the vertex and the 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 USB-6251 acquisition board that served for sound generation. The digitized signals were processed in a standard personal computer.

RESULTS

Baseline sessions

The animal first received two random-variation baseline sessions. These two sessions were followed by two fixed-stimulus baseline sessions.

In the random-variation baseline sessions, at the stimulus presentation rate of 15 s^{-1}, the number of pip trains in a trial varied from 75 (in 5 s stimuli) to 450 (in 30 s stimulus), mean of 263 trains. Respectively, in the fixed-stimulus sessions, each 2.9 s-long part of the stimulus contained 44 pip trains and every trial consisted of six fragments of different stimulus levels containing a total of 264 pip trains. The combination of the on-line and off-line averaging procedures allowed the measurement of brain responses to the test stimuli by averaging 1500 epochs in every session at every stimulus level both in random-variation and fixed-stimulus sessions (Fig. 4).

The records obtained in random-variation (Fig. 4A) and fixed-stimulus sessions (Fig. 4B) looked similar. They contained RFR as a rhythmic sequence of waves of the same frequency as the stimulation pip rate (1000 Hz). The RFRs featured a temporal lag relative to the stimulus (approximately 3 ms at the response start and 5 ms at the end), and their magnitude depended on the stimulus level.

In both kinds of sessions, RFR was indistinguishable from noise at a stimulus level of 90 dB re. 1 µPa rms and increased in magnitude with the stimulus level increase up to 115 dB re. 1 µPa rms. For quantitative evaluation of RFR magnitudes, the frequency spectra were computed as presented in Fig. 5. In both random-variation sessions (Fig. 5A) and fixed-stimulus sessions (Fig. 5B), RFR magnitude estimated by the 1 kHz spectrum peaks similarly featured dependence of magnitude on stimulus level.

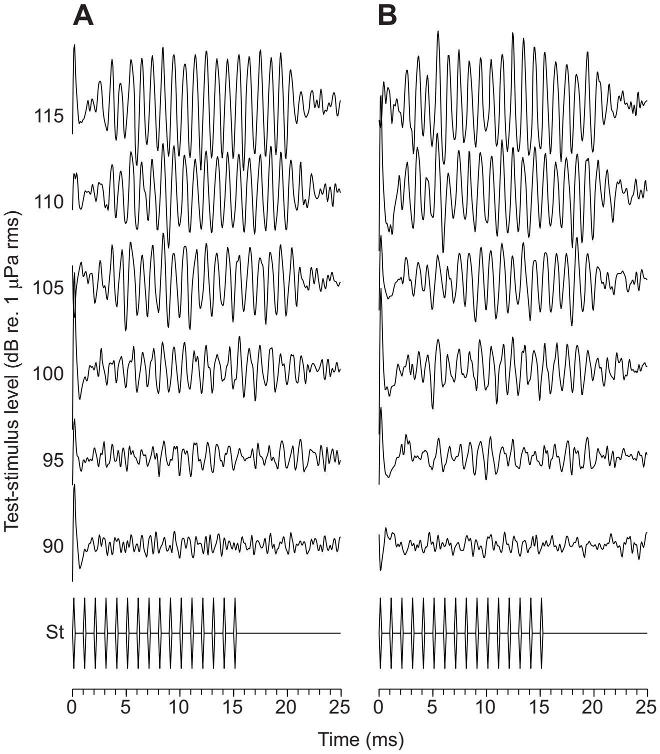

Fig. 4. **Example of rate-following response (RFR) records at various test-stimulus levels in baseline sessions.** (A) Random variation. (B) Fixed stimuli. Test-stimulus levels are indicated; St, stimulus (pip train) envelope. In both A and B, each record was obtained by averaging 1500 epochs. A zero-latency spike at the beginning of the records is an artifact produced by the board when it starts the acquisition process.

Conditioning sessions

There were 16 conditioning sessions. Four of the sessions were random-variation sessions, in which the animal could not anticipate when the loud sound would appear. The 12 subsequent sessions were fixed-stimulus sessions; in these sessions, the appearance of the loud sound was predictable.

Both in random-variation and fixed-stimulus sessions, the magnitude of the RFR produced by the test/warning stimuli was less than that seen in the baseline sessions at the same stimulus levels (Fig. 6). However, in the random-variation sessions, the reduction of RFR magnitude (Fig. 6A) was greater than in the fixed-stimulus sessions (Fig. 6B). This difference is more obvious when one compares the frequency spectra of the RFR records (Fig. 7). The same RFR magnitude appears at a stimulus level approximately 5 dB higher in the random-variation session (Fig. 7A) than in the fixed-stimulus session (Fig. 7B).

Quantitative comparison of baseline and conditioning data in random-variation and fixed-stimulus sessions

For a quantitative comparison, the RFR magnitudes were plotted as functions of stimulus level, as shown in Fig. 8 for several arbitrarily chosen sessions, one from each series: random-variation baseline, fixed-stimulus baseline, random-variation conditioning and fixed-stimulus conditioning. These functions were approximated by straight regression lines to obtain the threshold estimates. For the examples presented in Fig. 8, the threshold estimates (mean±s.e.)

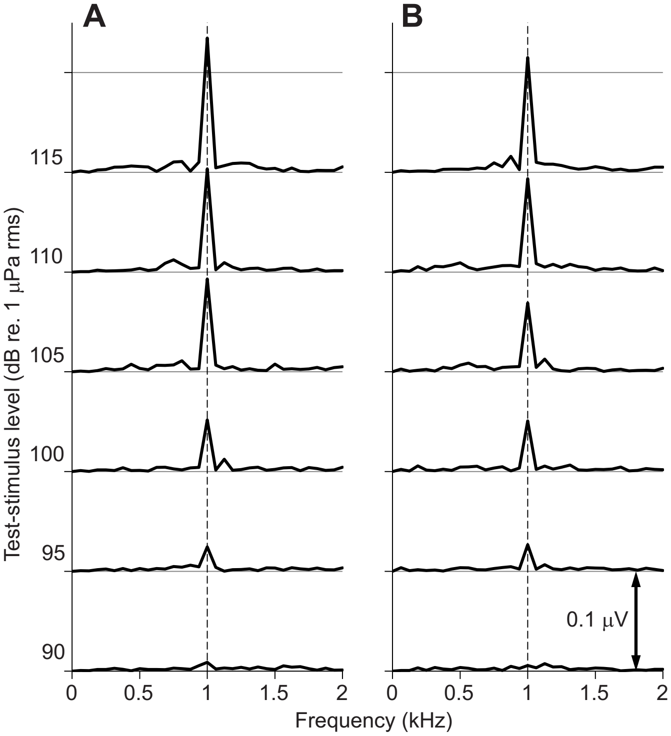

Fig. 5. **Frequency spectra of the records presented in Fig. 4.** A and B correspond to A and B, respectively, of Fig. 4.

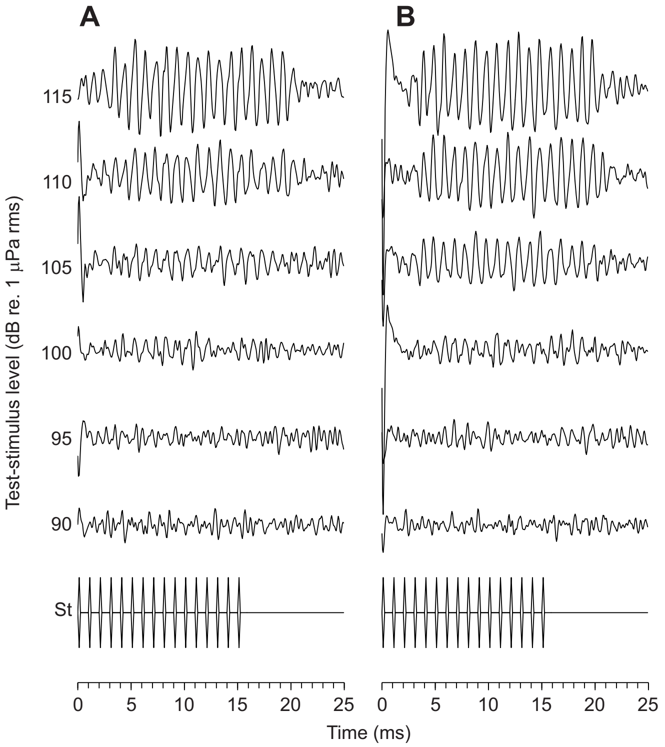

Fig. 6. Example of RFR records at various test-stimulus levels in conditioning sessions. (A) Random variation. (B) Fixed stimuli. Test-stimulus levels are indicated; St, stimulus (pip train) envelope. In both A and B, each record was obtained by averaging 1500 epochs.

were 89.3±1.1 dB for random-variation baseline, 88.1±0.5 dB for fixed-stimulus baseline, 99.5±0.6 dB for random-variation conditioning and 94.4±0.8 dB for fixed-stimulus conditioning sessions. The threshold estimates demonstrate a negligible (1.2 dB) difference between random-variation and fixed-stimulus baseline sessions, a substantial threshold increase by 10.2±1.3 dB in the random-variation conditioning session and a lesser threshold increase by 6.3±0.9 dB in the fixed-stimulus conditioning session.

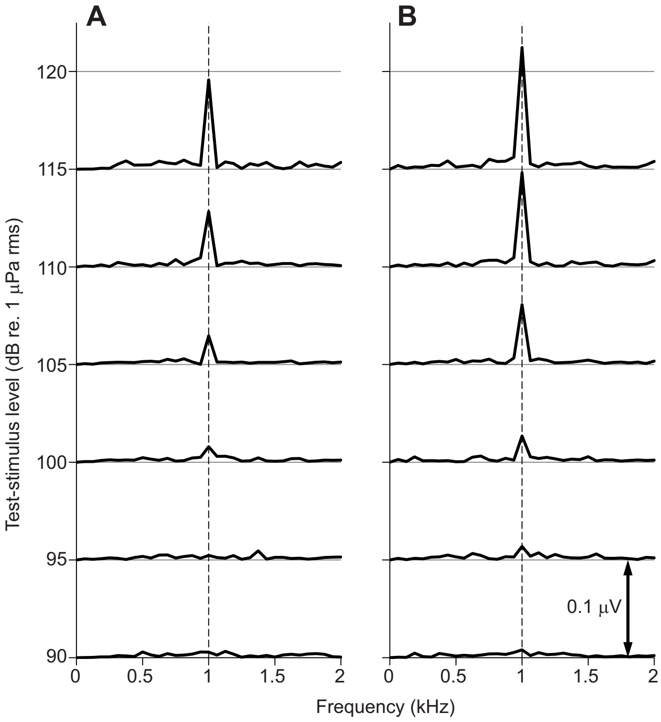

Fig. 7. Frequency spectra of the records presented in Fig. 6. A and B correspond to A and B, respectively, of Fig. 6.

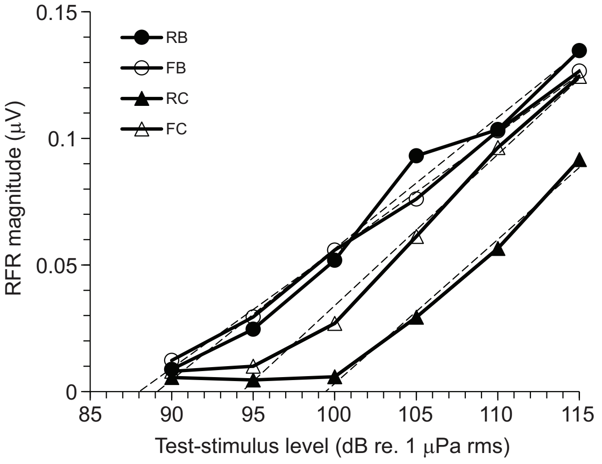

Fig. 8. RFR magnitude dependence on test (test/warning) signal level. RB, random-variation baseline; FB, fixed-stimulus baseline; RC, random-variation conditioning; FC, fixed-stimulus conditioning.

Using this procedure, thresholds were estimated consecutively in: (1) two random-variation baseline sessions, (2) two fixed-stimulus baselines sessions, (3) four random-variation conditioning sessions and (4) 12 fixed-stimulus conditioning sessions.

Different numbers of random-variation and fixed-stimulus sessions (4 and 12, respectively) were performed because the effect of randomly varied test/warning signals was previously investigated in detail in the same subject (Nachtigall and Supin, 2014, 2015). So, in the present study, the main attention was paid to the fixed-stimulus experimental design.

All the data are summarized in Fig. 9. Both random-variation and fixed-stimulus baseline sessions (nos 1–4 in Fig. 9) featured a minimum session-by-session scatter of threshold estimates, so these threshold estimates could be averaged with a mean of 88.6 dB re. 1 µPa rms. The first random-variation conditioning session (no. 5)

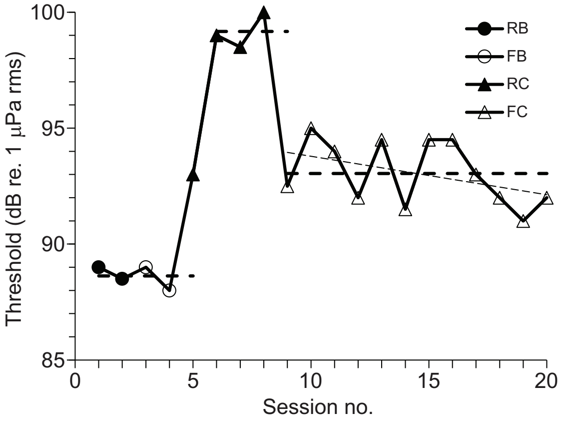

Fig. 9. Session-by-session threshold variation. RB, random-variation baseline; FB, fixed-stimulus baseline; RC, random-variation conditioning; FC, fixed-stimulus conditioning. Bold dashed lines indicate mean thresholds across baseline, random-variation conditioning and fixed-stimulus conditioning sessions. The thin dashed line is the regression line for thresholds across fixed-stimulus conditioning sessions.

featured a noticeable threshold increase (93.0 dB re. 1 μPa rms) with a further increase in subsequent random-variation conditioning session nos 6–8 with a mean of these three sessions of 99.2 dB re. 1 μPa rms, i.e. 10.6 dB above the mean baseline threshold. The change from random-variation to fixed-stimulus conditioning (session nos 9–20) resulted in a threshold decrease. The mean of the threshold estimates in 12 fixed-stimulus conditioning sessions was 93.0 dB re. 1 μPa rms, i.e. still higher than baseline thresholds by 4.4 dB but lower by 6.2 dB than the random-variation conditioning thresholds. Regression analysis of the threshold dynamics during the fixed-stimulus conditioning (session nos 9–20) revealed a slight tendency for session-by-session threshold decrease, although this tendency was not statistically significant (slope of −0.17±0.11 dB/session, mean±s.e., P=0.16).

DISCUSSION

As found previously, these data demonstrate that the dolphin dampened hearing sensitivity when a loud sound was preceded by a warning sound. The data further demonstrate that the ability of investigators to measure the amount of the hearing sensitivity shift may very well depend on when the animal expects the loud sound to occur.

The negative session-by-session trend of thresholds in the fixed-stimulus series shown in Fig. 9 is also noteworthy. This trend did not reach statistical significance. However, if one assumes that the trend takes place, it could mean that session-by-session the animal learned to more precisely adjust the dampening of her hearing to the moment of the onset of the loud sound. The thresholds were certainly not increased (or were increased less) in a larger part of the test/warning time, which resulted in session-by-session lowering of threshold estimates.

The most likely explanation for the difference between the random-variation and fixed-stimulus sessions is that, unlike the fixed-stimulus sessions, during the random-variation sessions the subject was not provided with exact information about the time of the loud sound onset. As the animal could not know when to mitigate the effect of the loud sound, she had to dampen her hearing sensitivity as soon as she heard the test/warning sounds. Thus, the hearing sensitivity was dampened during a larger part of the test/warning time, and the dampening effect was more readily detectable.

Based on the data and explanations presented above, it may be hypothesized that odontocetes are capable not only of learning to dampen their hearing when anticipating the appearance of a loud sound but also, when having the necessary information provided, of learning to adjust this dampening in such a manner as to keep their hearing sensitivity undamped, i.e. the audition effectively functioning, as long as possible.

The data also provide further evidence of the change being due to conditioning. The evidence follows from the fact that there was essentially the same mean amount and timing of loud sound energy presented to the animal in random-variation and fixed-stimulus sessions, and yet there was either a difference as high as 10.2 dB from baseline or a difference as low as 4.4 dB from baseline, depending on when the animal could expect the onset of the loud sound. This difference could not be explained from the point of view of direct (unconditioned) impact of the loud sounds on hearing sensitivity. Therefore, it is highly unlikely that the hearing change is due to a process like TTS. Alternatively, the conditioned nature of the observed dampening effect easily explains the difference between the random-variation and the fixed-stimulus sessions. In the fixed-stimulus sessions, the constant duration and the order of the level variation provided information of the precise instant when the loud sound would appear. Using this information, the animal could have learned when to expect the loud sound and to dampen its hearing immediately before onset of this sound. If so, hearing is dampened less during a part of the test/warning time, and the dampening effect is detected to a lesser extent.

The data presented in Fig. 9 show a rapid change with stimulus conditions immediately upon the change from one condition to the next, as seen in the results of session 9 compared with session 8. The animal apparently quickly altered her hearing change to the predictable time of arrival of the loud sound and therefore the dampening effect was not as observable as it had been in the random-variation situation. One must remember that the actual measure of hearing change is made prior to the loud sound. The quickness of this change may be explained by the fact that this animal was not naive to the hearing dampening experimental protocol and conditioning for hearing change occurs very rapidly. This dolphin had previously been exposed to warnings and loud sounds (Nachtigall and Supin, 2015) and had previously shown learned hearing sensation changes. Other work with a totally naive beluga whale (Nachtigall et al., 2016) demonstrated that the conditioning process for hearing change occurs very rapidly – within a 36 trial session.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
The authors declare equal contributions.

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