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Authors

Finneran, James J
Carder, Donald A
Schlundt, Carolyn E
et al.

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Temporary threshold shift in bottlenose dolphins (Tursiops truncatus) exposed to mid-frequency tones[a]

James J. Finneran[b] and Donald A. Carder
U.S. Navy Marine Mammal Program, Space and Naval Warfare Systems Center, San Diego, Code 2351, 53560 Hull Street, San Diego, California 92152

Carolyn E. Schlundt
EDO Professional Services, 3276 Rosecrans Street, San Diego, California 92110

Sam H. Ridgway
U.C. Veterinary Medical Center–San Diego, Department of Pathology, School of Medicine, University of California, La Jolla, California 92093-0612

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A behavioral response paradigm was used to measure hearing thresholds in bottlenose dolphins before and after exposure to 3 kHz tones with sound exposure levels (SELs) from 100 to 203 dB re 1 μPa^2 s. Experiments were conducted in a relatively quiet pool with ambient noise levels below 55 dB re 1 $\mu\text{Pa}^2/\text{Hz}$ at frequencies above 1 kHz. Experiments 1 and 2 featured 1-s exposures with hearing tested at 4.5 and 3 kHz, respectively. Experiment 3 featured 2-, 4-, and 8-s exposures with hearing tested at 4.5 kHz. For experiment 2, there were no significant differences between control and exposure sessions. For experiments 1 and 3, exposures with SEL=197 dB re 1 μPa^2 s and SEL$\geqslant$195 dB re 1 μPa^2 s, respectively, resulted in significantly higher TTS_4 than control sessions. For experiment 3 at SEL=195 dB re 1 μPa^2 s, the mean TTS_4 was 2.8 dB. These data are consistent with prior studies of TTS in dolphins exposed to pure tones and octave band noise and suggest that a SEL of 195 dB re 1 μPa^2 s is a reasonable threshold for the onset of TTS in dolphins and white whales exposed to midfrequency tones.

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I. INTRODUCTION

Certain anthropogenic underwater sounds, such as those produced by underwater explosions, seismic surveys, and military and oceanographic research sonars, have the potential to adversely affect marine animals (rev. Green *et al.*, 1994). Exposure to high intensity sound for a sufficient duration may result in physical injury to nonauditory structures such as the lungs and other gas-containing structures (Yelverton *et al.*, 1973; Dalecki *et al.*, 1999) and/or auditory effects such as a noise-induced threshold shift (NITS)—an increase in the auditory threshold after exposure to noise. A NITS may be permanent, called a permanent threshold shift (PTS), or temporary, called a temporary threshold shift (TTS).

Increased public concern and the application of the U.S. Marine Mammal Protection Act and Endangered Species Act to activities involving anthropogenic sound have resulted in a pressing need for specific information regarding safe limits for marine mammals exposed to underwater sound. Mass strandings of whales spatially and temporally coincident with the use of military midfrequency (generally from 1 to 10 kHz) tactical sonar (U.S. Department of Commerce and U.S. Department of the Navy, 2001; Jepson *et al.*, 2003) have further increased concern about the potential effects of intense sound on marine mammals and possible mitigation strategies. Since many marine mammals have sensitive hearing and rely upon underwater sound for communicating, foraging, and navigating, auditory effects such as a TTS or PTS are of particular concern.

Although there have been no efforts to directly measure PTS in marine mammals, a number of investigators have measured TTS. Schlundt *et al.* (2000) reported the results of TTS experiments conducted with five bottlenose dolphins (*Tursiops truncatus*) and two white whales (belugas, *Delphinapterus leucas*) exposed to 1-s tones at frequencies of 0.4, 3, 10, 20, and 75 kHz. At frequencies of 3, 10, and 20 kHz, sound pressure levels (SPLs) necessary to induce measurable amounts (6 dB or more) of TTS were between 192 and 201 dB re 1 μPa (mean of 195 dB re 1 μPa). Finneran *et al.* (2000; 2002b) conducted TTS experiments with dolphins and white whales exposed to impulsive sounds similar to those produced by distant underwater explosions and seismic waterguns. These studies showed that, for very short duration impulsive sounds, higher sound pressures were required to induce TTS than for longer duration tones. Nachtigall *et al.* (2003; 2004) measured TTS in a bottlenose dolphin exposed to octave-band noise centered at 7.5 kHz. Exposures with

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[a] Portions of these data were presented at the 142nd Meeting of the Acoustical Society of America [Finneran, *et al.* (2001). "Temporary threshold shift (TTS) in bottlenose dolphins (Tursiops truncatus) exposed to tonal signals," J. Acoust. Soc. Am.**110**, 2749(A)] and 15th Biennial Conference on the Biology of Marine Mammals [Finneran, *et al.* (2003). "Auditory effects of mid-frequency tones on cetaceans"].

[b] Electronic mail: james.finneran@navy.mil

SPLs of 160 dB re 1 μPa and durations of 30–50 minutes induced 5–8 dB of TTS measured 5 min after exposure. Kastak *et al.* (1996) reported TTS in a harbor seal (*Phoca vitulina*) exposed to airborne noise from nearby construction. Kastak *et al.* (1999) reported TTS in a California sea lion (*Zalophus californianus*), harbor seal, and Northern elephant seal (*Mirounga angustirostris*) exposed to underwater octave band noise. Finneran *et al.* (2003) exposed California sea lions to single underwater impulses produced from an arc-gap transducer, but found no measurable TTS at SPLs up to 178–183 dB re 1 μPa.

Because Schlundt *et al.* (2000) used tones similar to sonar pings, these data are the most directly applicable to military tactical sonars. However, by necessity this study was conducted in San Diego Bay, where ambient noise levels are relatively high and quite variable. For this reason, Schlundt *et al.* employed broadband masking noise to provide a "floor effect" and keep thresholds consistent from session to session despite variations in ambient noise levels. Specific tests to determine the effects, if any, of the masking noise were inconclusive (Schlundt *et al.*, 2000); however, terrestrial mammal data suggest that the presence of masking noise would result in smaller observed TTS (Parker *et al.*, 1976; Humes, 1980).

This paper presents the results of three TTS experiments with bottlenose dolphins exposed to 3-kHz tones. This particular frequency was selected because it falls within the range used by the U.S. Navy's AN/SQS-53 mid-frequency tactical sonar (U.S. Department of Commerce and U.S. Department of the Navy, 2001). The first two experiments were designed to address lingering questions regarding the potential effects of masking noise used by Schlundt *et al.* (2000) by conducting tests in a pool with low ambient noise level and no masking noise. The third experiment was designed to investigate the effects of tone duration and the growth of TTS with increasing exposure.

II. METHODS

A. Subjects

Subjects were two male bottlenose dolphins: BEN (estimated age 38–40 yr during the study, approximately 250 kg) and NAY (18–20 yr, approximately 255 kg). Both subjects had substantial prior experience in cooperative psychophysical testing, including hearing tests. Subjects were housed in floating netted enclosures 9×9 to 12×24 m located in San Diego Bay, CA. The study followed a protocol approved by the Institutional Animal Care and Use Committee at The Space and Naval Warfare Systems Center, San Diego. Both BEN and NAY were tested during experiments 1 and 2; only BEN was tested during experiment 3.

B. Hearing test apparatus

Figure 1 shows the experimental configuration. Tests were conducted in an above-ground, 6.1-m-diam, 1.5-m-deep circular, vinyl-walled pool. Figure 2 compares ambient noise levels in the test pool and San Diego Bay measured with a low-noise hydrophone (Reson TC 4032). The San Diego Bay data are mean noise levels over a one-month period. The pool data are mean levels from a single measurement (100 averages, 195-Hz frequency resolution) and are representative of typical mean ambient noise levels in the pool. At frequencies above approximately 1 kHz, mean ambient noise pressure spectral density levels were below 55 dB re 1 $\mu Pa^2/Hz$.

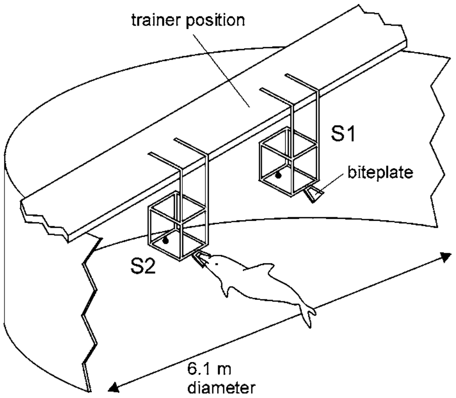

FIG. 1. Experimental setup for hearing tests and sound exposures.

A wooden deck located above the pool supported two "listening stations," designated as the "S1 station" and the "S2 station." The S1 station was the site for the presentation of the fatiguing sound exposure and a "start" signal to begin the hearing test. Hearing tests were conducted at the S2 station. Two stations were used to physically separate the site of the fatiguing sound exposure from the hearing test location. Each station consisted of a PVC frame with a plastic "biteplate" upon which the subjects were trained to position. The S1 and S2 biteplates were located at mid-depth. Each station contained an underwater video camera; a third video camera was located in-air with a view of both stations.

The S1 station contained an underwater sound projector (ITC 1042 or ITC 1032) used to produce a 1-s sinusoidal amplitude modulated tone as a signal for the subject to begin hearing tests. These start tones, or "S1 tones," had a carrier frequency of 12 kHz, modulation frequency and depth of 700 Hz and 100%, respectively, and a SPL of approximately 100 dB re 1 μPa. S1 tones were generated using a PC containing a multifunction data acquisition board (National Instruments

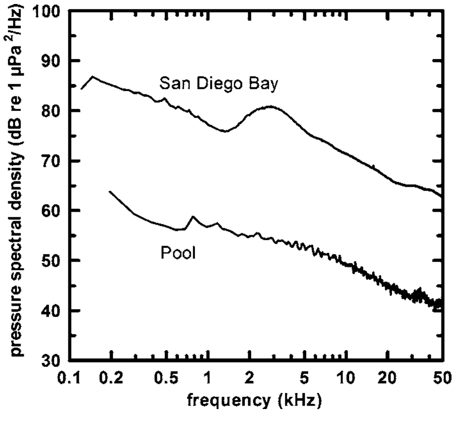

FIG. 2. Ambient noise levels measured in test pool and San Diego Bay.

TABLE I. Exposure and hearing test parameters for experiments 1–3.

Experiment	Exposure frequency (kHz)	Exposure SPL (dB re 1 μPa)	Exposure duration (s)	Hearing test frequency (kHz)
1	3	100–200	1	4.5
2	3	180–200	1	3
3	3	144–195	2–8	4.5

PCI-MIO-16E-1), filtered (Ithaco 4302), and amplified (HP 467A or BGW PS 4) before being input to the S1 sound projector.

The S2 station contained an underwater sound projector (ITC 1001 or ITC 1032) used to generate hearing test tones, or "S2 tones." The S2 tones had frequencies of 4.5 kHz (experiments 1 and 3) and 3 kHz (experiment 2) and durations of 500 ms, including 50-ms rise and fall times. S2 tones were digitally generated (PCI-MIO-16E-1), attenuated (Tucker-Davis PA4, PA5), filtered (Ithaco 4302), and amplified (BGW PS2 or PS4) before being input to the sound projector. The sound pressure during each S2 tone presentation was measured using a hydrophone (B&K 8105 or Reson TC4033) located near the S2 biteplate. The hydrophone signal was filtered and amplified (B&K 2692), digitized (PCI-MIO-16E-1), and stored on the PC. The time each S1 and S2 tone was presented was recorded and stored on the PC. Custom software (Finneran, 2003) was used to generate, record, and calibrate hearing test tones and to record the subjects' responses during hearing tests.

The small volume of the test pool resulted in large spatial variations in sound pressure. To mitigate this effect, S2 tone SPLs were calibrated before each session using two hydrophones (B&K 8105) positioned at the approximate locations of the subjects' ears (without the subject present). The hydrophone signals were filtered and amplified (B&K 2692) before being digitized (PCI-MIO-16E-1). Hearing test tone levels were estimated using the (incoherent) mean SPL from the two hydrophones. This gave an estimate of the spatial average of the SPL on a scale comparable with the subject's head.

C. Fatiguing stimuli

Fatiguing stimuli were generated using a piezoelectric cylinder (ITC 2015) positioned approximately 1 m in front of the S1 biteplate. Fatiguing stimuli were digitally generated (PCI-MIO-16E-1), attenuated (Tucker-Davis PA4 or PA5), filtered (Ithaco 4302), amplified (BGW PS4 or Crown Macro-Tech 2400), and input to the sound projector. Fatiguing stimuli were 3-kHz tones with rise and fall times of 100 ms, total durations (including the rise and fall) from 1 to 8 s, and SPLs from 100 to 200 dB re 1 μPa (depending on the particular experiment). Table I lists the fatiguing stimulus parameters and S2 frequencies for the three experiments. Exposure levels began at low levels and gradually increased during experiment 1; during experiments 2 and 3 exposure levels (and durations) were tested in quasirandom sequences.

Fatiguing sound levels for experiments 1 and 2 were estimated from calibration measurements conducted without the subject present. Instantaneous sound pressures were measured using two hydrophones (B&K 8105) positioned at the approximate locations of the subjects' ears when on the S1 biteplate. The hydrophone signals were filtered and amplified (B&K 2635 and Krohn-Hite 3C series modules), and digitized (PCI-MIO-16E-1). Since each subject normally left the S1 station before cessation of the fatiguing stimulus, and sound pressures spatially varied in the pool, calibration levels for experiments 1 and 2 must be considered as only estimates of the actual exposure. During experiment 3, the instantaneous sound pressure during the actual fatiguing exposure was measured from two suction cup-mounted hydrophones (B&K 8105) worn by the subject. The hydrophones were located near the left and right external auditory meatus. This allowed a more meaningful estimate of the actual received sound level during the exposure.

For all experiments, custom software was used to calculate the SPL and (unweighted) sound exposure level (SEL) from the digitized pressure waveforms. SEL was calculated using

$$\mathrm{SEL} = 10 \log_{10}\left(\frac{\int_0^T p^2(t)dt}{P_0^2 T_0}\right), \qquad (1)$$

where $p(t)$ is the instantaneous sound pressure, $P_0 = 1$ μPa, and $T_0 = 1$ s [American National Standards Institute (ANSI), 1994]. Numeric values and units for SEL are equivalent to those of the energy flux density (EFD) level metrics used by previous authors (e.g., Finneran *et al.*, 2000; 2002b; Finneran *et al.*, 2003), where EFD level was expressed in decibels relative to the EFD of a plane wave with rms pressure P_0 and duration T_0, in the same environment (Marshall, 1996). Exposure levels were defined using the mean values of the SPL and SEL measurements from the two hydrophones. It was common during experiment 3 for the SPL/SEL measured during the exposure to differ from the desired values by 1–3 dB. For analysis purposes, exposures were placed into discrete SPL and SEL groups. Most analyses were based on the SEL of the exposure (regardless of the SPL/duration that produced that SEL).

D. Procedure

1. Overview

A single "exposure session," featuring an exposure to a fatiguing stimulus, or "control session," where the fatiguing sound exposure was simulated but no intense sound was actually produced, was conducted each test day. The control sessions were randomly interspersed with the exposure sessions; exposure sessions never occurred on consecutive days. A hearing test was performed before the exposure (or mock exposure) to provide the subject's pre-exposure hearing threshold. Another hearing test was conducted after the exposure (or mock exposure) to provide the post-exposure hearing threshold. The amount of TTS was determined by subtracting the pre-exposure threshold from the post-exposure threshold. Total session time was approximately 45 min.

2. Pre-exposure hearing tests

The hearing test procedure was based on the Method of Free Response, or MFR (Egan *et al.*, 1961) and was similar to that used by Schlundt *et al.* (2000) and Finneran *et al.* (2000; 2002b; 2003). Each hearing test consisted of a number of relatively long observation periods, or "dives," during which the subject was presented with a number of hearing test tones. Each dive began with the trainer directing the animal (with a hand signal) to the S1 station. The subject was trained to remain on the S1 station until presented with the S1 start signal, which signaled the subject to proceed to the S2 station. Once the subject was positioned at the S2 station, a block of hearing test trials was presented. The trial block was ended when the trainer sounded an underwater buzzer to signal the subject to return to the surface and receive fish reward. The process was then repeated as necessary.

Each trial block contained a variable number of 2-s duration trials. The time interval between trials, defined from the start of one trial to the start of the next trial, was fixed at 4 s. Fifty-percent of the trials contained an S2 tone beginning 50 ms before the trial start. The remaining 50% of the trials were no-tone or "catch" trials. Since the subject was not notified at the start of each trial, the catch trial periods functioned as "equipment catch trials" and were primarily used to confirm that the sound system was not producing artifacts coinciding with the stimulus. Subjects were trained to whistle if they heard a tone and to remain quiet otherwise. The first S2 tone was presented approximately 10 dB above the baseline threshold. The amplitudes of the S2 tones were decreased 2 dB following each hit and increased 2 dB following each miss (e.g., Cornsweet, 1962).

The false alarm rate R_{FA} was defined as

$$R_{\mathrm{FA}} = \frac{N_{\mathrm{FA}}}{T - N_{\mathrm{S2}} T_1} T_1, \tag{2}$$

where N_{FA} is the number of false alarms (whistles occurring during no-tone trials or between trials), T is the total amount of time the subject spent on the S2 station, N_{S2} is the number of S2 tone trials presented, and T_1 is the trial duration. The denominator of Eq. (2) is the total amount of time during which the subject was on the S2 station without a tone trial present, therefore R_{FA} is a way of normalizing the number of false alarms with respect to the amount of time that the subject had an opportunity to commit a false alarm (see Finneran *et al.*, 2002a; 2002b). This study employed a modified version of the MFR, so the R_{FA} values calculated here are not identical to those obtained with the MFR or a single interval experiment; however, they do enable one to assess a subject's response bias from session to session.

A randomized schedule was used to determine the point at which reinforcement was delivered (i.e., the number of trials per dive). Dives were concluded following a number of correct responses (i.e., either hits or correct rejections) randomly chosen from a predefined schedule. The number of required correct responses varied from 4 to 8. Dive times were normally kept to less than 2 min. The amount of reward was scaled to the performance of the subject during the dive (e.g., more reinforcement was given for more correct responses) by assigning individual scores for hits, misses, correct rejections, and false alarms and summing the scores for each dive. The dive score was multiplied by a subject-specific scale factor to obtain a number representing the amount of fish to be given. To maintain each subject's performance, periodic (about once/week) training sessions were conducted where responses to low-level tones (i.e., at a lower level than any previously responded to) were preferentially reinforced. Training sessions did not feature fatiguing stimulus exposures and were not used to estimate audiograms.

After reinforcement, the next dive was begun and the procedure repeated until the hearing test was complete. Pre-exposure hearing thresholds were based on the last 10 hit/miss and miss/hit "reversal" points from the staircase. The pre-exposure threshold could usually be estimated after 2–5 dives or 25–30 trials. Pre-exposure thresholds were required to be within ±5 dB of established baseline values for fatiguing sound exposures to occur. Baseline threshold standard deviations were 2–3 dB at 3 and 4.5 kHz; less than 10% of sessions were excluded for failure for pre-exposure thresholds to occur within ±5 dB of baseline values.

3. Post-exposure hearing tests

The post-exposure hearing test procedure was identical to the pre-exposure procedure with two exceptions: (1) During exposure sessions, the fatiguing stimulus was presented instead of the first S1 start tone of the post-exposure hearing test. (2) The post-exposure hearing tests were conducted for at least 10 minutes to enable any NITS to be tracked over time.

The post-exposure hearing test resulted in a record of the subject's performance (hit or miss) for each tone trial. These data were then converted to a series of reversals. The time and amplitude of each reversal were defined as the mean time and mean SPL, respectively, of the hit/miss pair. The hearing threshold as a function of the time post-exposure was estimated by applying a 10-point moving average to the set of reversals. Each output of the moving average consisted of the mean SPL over the 10 reversals and the mean time over which the 10 reversals occurred. Thresholds at specific times post-exposure were obtained by interpolating within the collection of thresholds from the moving average. The amount of TTS 4 min (TTS_4) and 10 min (TTS_{10}) after exposure were used to compare the results of the different exposure conditions. For this study, 4 min was about the shortest time in which a threshold could reliably be obtained after exposure. Four minutes is commonly used as an early TTS measurement time; measurements before 2 min may be susceptible to the "bounce" phenomenon observed in terrestrial mammals and result in less observed TTS than at later times (Hirsh and Ward, 1952; Hirsh and Bilger, 1955). For the specific reinforcement schedules and available food per session, TTS_{10} was found to be a practical upper limit for the maximum time for TTS measurement after exposure. TTS_4 was obtained in all but one of the exposure and control sessions: for subject NAY exposed to a 200 dB re 1 μPa tone during experiment 1, the first post-exposure threshold was not obtained until 7.2 min after the exposure.

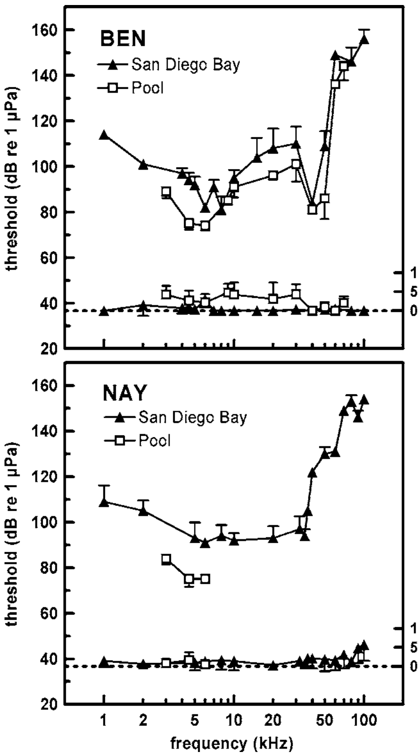

FIG. 3. Behavioral hearing thresholds and false alarm rates for subjects BEN (upper panel) and NAY (lower panel) measured in test pool and San Diego Bay. Symbols represent mean values and error bars indicate one standard deviation.

Previous studies of TTS in marine mammals have reported, in addition to auditory effects of noise exposure, effects on the behavior of the subjects. Specific behavioral effects most often reported can be broadly described as attempts by the subjects to leave the area of a continuing exposure (e.g., Kastak *et al.*, 1999) or to avoid the site of previous exposures (Finneran *et al.*, 2000; Schlundt *et al.*, 2000; Finneran *et al.*, 2002b). Behavioral reactions observed during experiments 1 and 2 have been previously reported (Finneran and Schlundt, 2004). Behavioral reactions of BEN during experiment 3 were analyzed using the same approach used in Finneran and Schlundt (2004). Observations of the subject's behavior during training, control, and exposure sessions were used to subjectively grade each exposure session as "normal behavior" or "altered behavior." The most common example of altered behavior was a reluctance to return to the S1 station on the dive immediately following the fatiguing sound exposure. For each exposure SEL, the percentage of sessions with altered behavior was calculated.

III. RESULTS

A. Baseline hearing thresholds and ambient noise levels

Figure 3 shows hearing thresholds and false alarm rates measured for BEN (upper panel) and NAY (lower panel) in San Diego Bay and in the test pool. Thresholds were measured in the pool before experiments 1–3; the data were pooled to create Fig. 3. NAY was only tested in the pool at frequencies near the fatiguing stimulus and hearing test tone frequencies. The number of replications at each frequency varied: for tests in San Diego Bay, between 2 and 9; in the test pool, between 1 and 28, depending on frequency (most tests were conducted at 3, 4.5, and 6 kHz). Although both subjects had poor sensitivity above 40–50 kHz, within the range of 3–4.5 kHz sensitivity was comparable to that measured for bottlenose dolphins by Johnson (1967) and suggests "normal" hearing at these frequencies. Both subjects were relatively conservative and did not commit large numbers of false alarms, especially when tested in San Diego Bay.

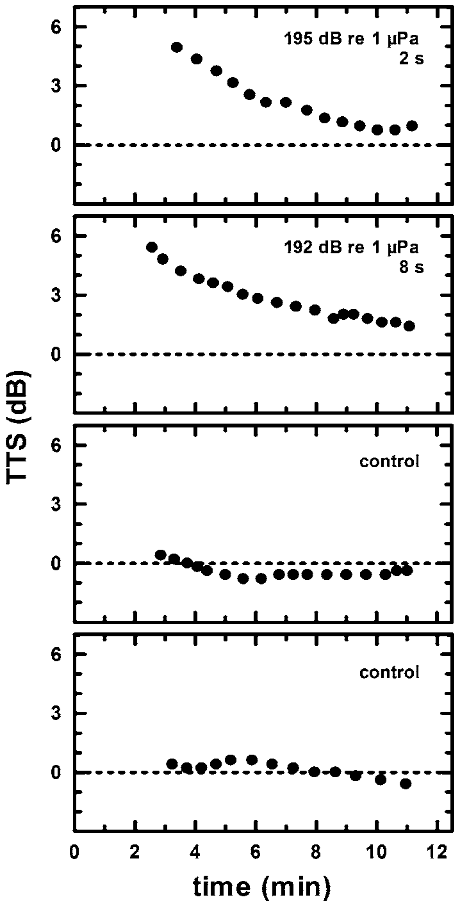

FIG. 4. Examples of TTS measured as a function of time post-exposure for exposure and control sessions.

B. TTS Growth and recovery

Figure 4 shows four examples of the measured TTS as a function of time post-exposure. The upper two panels show the results of exposure sessions with SPLs and durations of 195 dB re 1 μPa, 2 s and 192 dB re 1 μPa, 8 s, respectively. The lower two data sets are from control sessions. The patterns seen in the upper two panels were common—an initial shift that decreased with increasing time post-exposure. In most cases (88% of exposure sessions) TTS_{10} was less than 3 dB. Most exceptions occurred at the higher exposure conditions. For example, at exposure SELs of 201 and 203 dB re 1 $\mu\mathrm{Pa}^2$ s, two-thirds of the exposures resulted in $\mathrm{TTS}_{10} > 3$ dB; the mean values for TTS_{10} at these exposure levels were 3.8 and 4.3 dB, respectively. Variation of 2–4 dB was common in the control sessions and was consistent with the

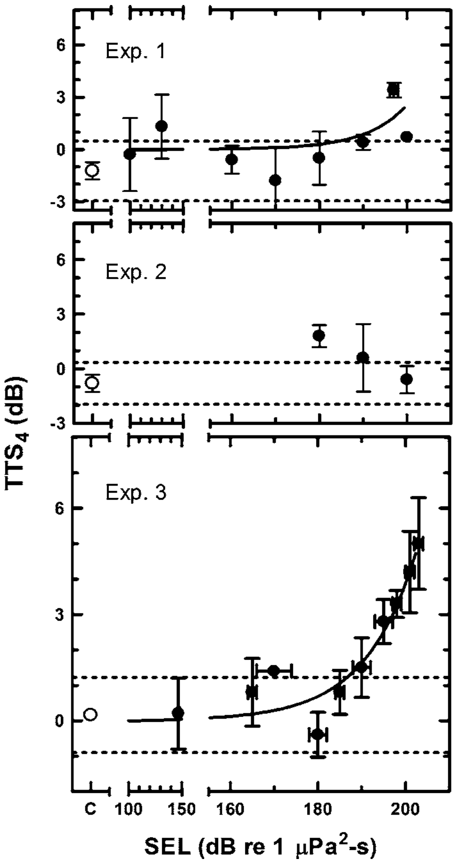

FIG. 5. Growth of TTS$_4$ as a function of exposure SEL for experiments 1 (upper), 2 (middle), and 3 (lower). The symbols and vertical error bars represent the mean and the standard error of the mean, respectively. The horizontal error bars indicate the range of exposure SELs grouped together for analysis. The open circles indicate the mean TTS$_4$ measured during control sessions. The dashed lines represent the control mean±one standard deviation. The solid lines in the top and bottom panels were generated by performing a nonlinear regression with an exponential growth equation and are intended to be a visual aid only.

normal variation seen in thresholds measured using behavioral response paradigms [e.g., Johnson, 1967; National Institute for Occupational Safety and Health (NIOSH), 1998)]. There were no permanent shifts in either subjects' hearing thresholds.

Figure 5 summarizes the measured TTS$_4$ as a function of SEL for experiments 1 (upper), 2 (middle), and 3 (lower), respectively. Results were pooled from both subjects in experiments 1 and 2. A one-way ANOVA with Dunnett's post-test (GraphPad Software, 2003) was performed to test for significant differences between the mean values of TTS$_4$ measured during control and exposure sessions within each experiment. For experiment 1, significant differences existed between the control data and exposures with SEL=197 dB re 1 μPa^2 s ($p<0.01$). The ANOVA did not include the point at SEL=200 dB re 1 μPa^2 s, since only one value of TTS$_4$ was obtained (TTS$_4$ may have been larger than the TTS measured 7.2 min post-exposure in the second exposure at this SEL). For experiment 2, there were no significant differences between control and exposure results ($p>0.05$). For experiment 3, significant differences existed for all exposures with SEL$\geqslant$195 dB re 1 μPa^2 s ($p<0.01$). For experiment 3 at SEL=195 dB re 1 μPa^2 s, the mean TTS$_4$ was 2.8 dB. Exposure SPLs resulting in statistically significant differences ($p<0.01$) between control and exposure sessions were 197 dB re 1 μPa (1 s), 190 and 195 dB re 1 μPa (2 s), 184, 190, and 195 dB re 1 μPa (4 s), and 190, 192, 195 dB re 1 μPa (8-s exposure).

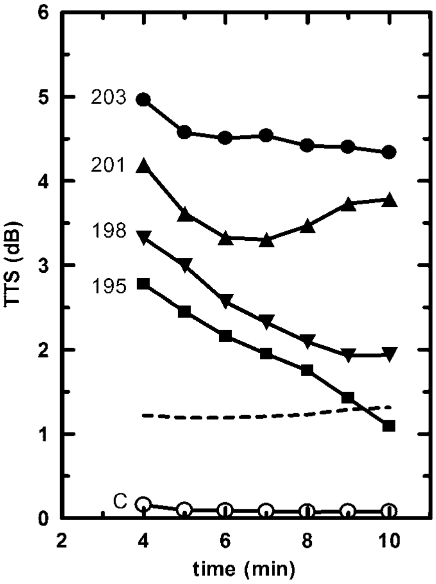

FIG. 6. Recovery of TTS for exposures producing statistically significant TTS$_4$. The numbers by each series indicate the exposure SEL in dB re 1 μPa^2 s. The open circles show the mean values of TTS for the control sessions; the dashed line is the control mean plus one standard deviation. Standard deviations for the exposure TTSs were generally 1–2 dB and, to preserve visual clarity, are not shown.

Figure 6 shows the mean values of TTS measured between 4 and 10 min post-exposure for exposure SELs of 195 to 203 dB re 1 μPa^2 s (the exposure SELs producing statistically significant amounts of TTS$_4$). The measured TTS generally decreased with increasing time post-exposure. For SELs$<$200 dB re 1 μPa^2 s, TTS$_{10}$ was less than approximately 2 dB and was not significantly different than the control session mean TTS$_{10}$. Exposures with higher SELs produced larger amounts of TTS$_4$ and subsequently required longer times for recovery. For SELs of 201 and 203 dB re 1 μPa^2 s, recovery was not complete by 10 min. In all cases, recovery to within the normal range of pre-exposure thresholds was complete by the next testing day (generally the next calendar day).

C. Behavioral results

Figure 7 shows the percentage of sessions with "altered behavior." The same trends reported by Finneran and Schlundt (2004) exist: little or no changes in behavior at low exposure levels and an increasing frequency of behavioral changes as the exposure level increased. The sparse data prevented fitting a sigmoidal dose-response curve.

D. Relationship to previous studies

The results of the present study were combined with the 3-, 10-, and 20-kHz TTS data from Schlundt *et al.* (2000) to create a dose-response curve for the occurrence of TTS in dolphins and white whales exposed to mid-frequency tones.

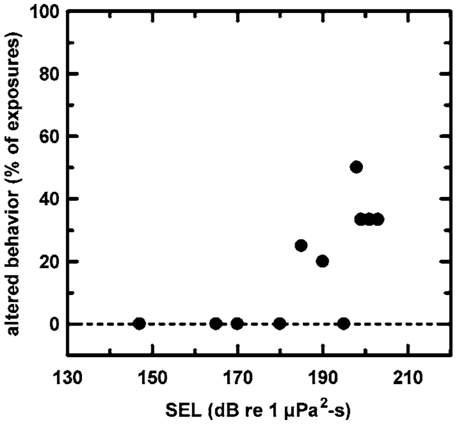

FIG. 7. Percentage of fatiguing sound exposure sessions with altered behavior as a function of exposure SEL for experiment 3.

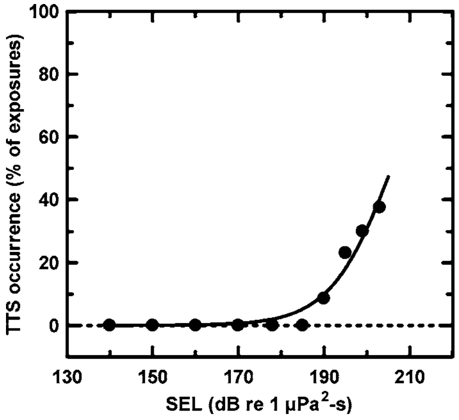

FIG. 8. Pooled data from Schlundt *et al.* (2000) and the present study showing the percentage of exposures resulting in detectable TTS (Schlundt *et al.*, 6 dB or more; present study, 2.8 dB or more) as a function of exposure SEL.

This required the result of each sound exposure, normally described by the amount of TTS, to be converted to a binary result indicating "TTS" or "no TTS." Since Schlundt *et al.* (2000) used a 6-dB criterion for the occurrence of TTS, sound exposures from Schlundt *et al.* producing 6 dB or more TTS were classified as TTS and those producing less than 6 dB TTS were categorized as no TTS. For the present study, data from experiment 3 revealed that exposures with SEL$\geqslant$195 dB re 1 μPa^2 s produced statistically significant amounts of TTS. The mean amount of TTS at SEL =195 dB re 1 μPa^2 s was 2.8 dB, therefore, for the present study, 2.8 dB of TTS was selected as the criterion: Individual exposures resulting in 2.8 dB or more TTS were classified as causing TTS; those producing less than 2.8 dB of TTS were considered to result in no TTS. The data were pooled by exposure SEL. Exposures with SELs (in dB re 1 μPa^2 s) within the following ranges were assigned the nominal SEL listed in parentheses: 100 (100), 125–134 (130), 135–144 (140), 145–154 (150), 155–164 (160), 165–174 (170), 175–182 (178), 183–187 (185), 188–192 (190), 193–197 (195), 198–202 (200), and 203–204 (203). For each exposure SEL group having more than two members (all but the 100 and 130 dB re 1 μPa^2 s groups), the percentage of exposures resulting in TTS was calculated. Figure 8 shows the resulting occurrence of TTS (percentage of exposure sessions resulting in TTS) as a function of exposure SEL. A nonlinear regression was used to fit a four-parameter logistic model to the data (GraphPad Software, 2003) in order to generate a smooth dose-response curve.

Figure 9 compares exposure levels necessary to cause measurable amounts of TTS from the present study to those previously published for cetaceans (Finneran *et al.*, 2000; 2002b; Schlundt *et al.*, 2000; Nachtigall *et al.*, 2004). The solid line in the middle panel of Fig. 9 has a slope of -3 dB per doubling of time and passes through the point where SPL is 195 dB re 1 μPa [the mean SPL required for measurable TTS from Schlundt *et al.*, (2000)] and the exposure duration is 1 s. This type of line is sometimes referred to as an "equal energy line," because all points on the line have the same sound exposure, which for a plane progressive wave is proportional to sound energy flux density. This line appears in the lower panel as a horizontal line at 195 dB re 1 μPa^2 s.

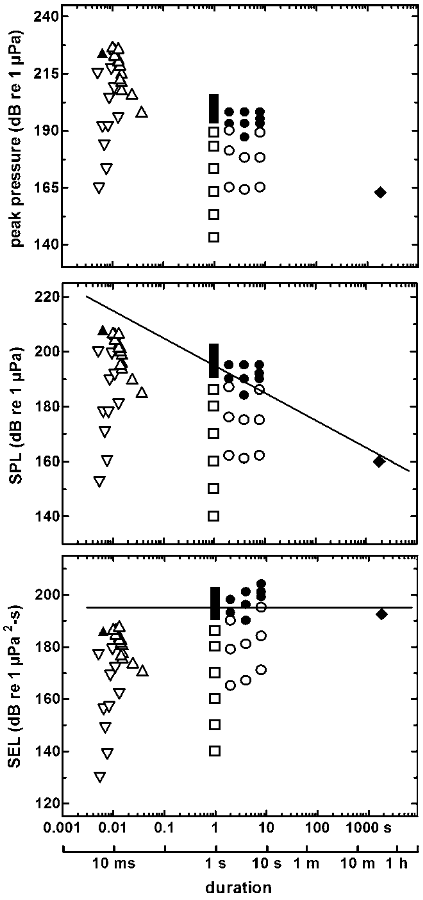

FIG. 9. Summary of bottlenose dolphin and white whale TTS experimental data. Individual exposures are shown in terms of peak pressure (upper panel), SPL (middle panel), and SEL (lower panel) vs exposure duration. Closed symbols indicate exposures where measurable TTS was observed; open symbols represent exposure conditions that did not produce TTS. The circles indicate the results of the present study. The triangles represent impulsive test results from Finneran *et al.* (2000; 2002b). The squares show the 3-, 10-, and 20-kHz data from Schlundt *et al.* (2000). The diamond represents data from Nachtigall *et al.* (2003; 2004). Peak pressures from Nachtigall *et al.* (2003; 2004) were approximated as the (rms) SPL+3 dB. The solid lines represent an "equal-energy" condition.

IV. DISCUSSION

A. Testing environment and baseline hearing thresholds

One of the main motivations for this study was to determine the effects, if any, of the masking noise employed by Schlundt *et al.* (2000) while measuring TTS in San Diego Bay. To accomplish this, tests were conducted in an aboveground pool with low, relatively constant ambient noise levels, therefore no intentional masking noise was introduced. Because of the small volume of the pool, large spatial variations in sound pressure were observed, making calibration of hearing test tones difficult. To overcome this, two hydrophones were used to provide an average sound pressure over a spatial scale comparable to the size of the subjects' heads. This lessened the chance of sound pressure peaks or nulls giving a false impression of the SPL actually received by the subject and allowed consistent hearing threshold measurements from day-to-day.

The spatial variations in sound pressure also affected estimates of the received levels during the fatiguing sound exposure. During experiments 1 and 2, fatiguing levels were estimated from two (stationary) sound pressure measurements made without the subject present. Because the subjects moved away from the exposure site toward the hearing test location shortly after the exposure start, exposure levels for experiments 1 and 2 must be considered as only approximate. The actual exposures may have been higher or lower (perhaps up to 4–5 dB), depending on the subject's path and the spatial variation in the sound field. For experiment 3, a better representation of the received sound level was obtained by having the subject wear hydrophones mounted on suction cups. The hydrophones were positioned to place the receiving element near each external auditory meatus, in order to provide an estimate of the sound pressure near the ear during the actual exposure—a sort of "acoustic dosimeter." As a result, the instantaneous sound pressure received by the subject was estimated and the desired quantities (SPL and SEL) calculated. In many cases the measured SPL and SEL differed from the intended exposures by 1–3 dB and replications could not be obtained with exactly the same exposure levels, forcing data to be consolidated into groups of similar exposure level for analysis.

Because of time constraints, detailed audiograms were not obtained for both subjects in the pool, and the audiograms in Fig. 3 are primarily intended to show the hearing sensitivity of each subject near the exposure and hearing test frequencies. Both subjects had relatively poor high-frequency hearing (above 40–50 kHz), which may be relatively common for adult male dolphins (Ridgway and Carder, 1997; Moore *et al.*, 2004). Sensitivity of both subjects within the range of 3 to 4.5 kHz was comparable to values commonly reported for bottlenose dolphins (e.g., Johnson, 1967) and suggests "normal" hearing at the exposure and hearing test frequencies. There are no data to indicate that the amount of TTS resulting from a narrow-band exposure is affected by hearing loss at frequencies outside the range where TTS would is expected to occur. Therefore, even though both subjects had pre-existing high-frequency hearing loss (above 40–50 kHz), this should not have affected the amount of TTS produced by exposures at much lower frequencies (3 kHz) where their hearing sensitivities appeared normal.

B. Growth of TTS

Figure 5 summarizes the growth of TTS with increasing SEL observed during experiments 1–3. The most important results were obtained in experiment 3, where the exposure SELs and relatively large number of exposure sessions enable more definitive conclusions. Over the range of exposures tested, the amount of TTS was correlated with and increased monotonically with increasing SEL. Statistically significant amounts of TTS were observed for exposure SELs $\geqslant 195$ dB re 1 μPa^2 s. This is identical to the mean SEL for causing "onset-TTS," defined as 6 dB or more of TTS by Schlundt *et al.* (2000) for 3-, 10-, and 20-kHz exposures. It is also very close to the SELs of 193-194 dB re 1 μPa^2 s reported by Nachtigall *et al.* (2004) for a bottlenose dolphin exposed to long duration octave band noise. The data from the present study also suggest that the masking noise employed by Schlundt *et al.* (2000) did not have a substantial effect on the onset-TTS levels observed.

An important application of marine mammal TTS data is to estimate exposure levels that may cause PTS. This requires an estimate for the rate of TTS growth with exposure—how much additional TTS is produced by increases in exposure level. At the highest SEL tested in this study (203 dB re 1 μPa^2 s), the slope of the curve in the lower panel of Fig. 5 is approximately 0.4 dB/dB, indicating that each additional dB of SEL would produce an additional 0.4 dB of TTS_4. This is much lower than related quantities reported in humans ($\sim$1.6 dB TTS_2/dB SEL, Ward *et al.*, 1958; 1959) after exposures of 12–102 min causing TTS_2 up to 30–40 dB. Because the slopes of the exponential curves in Fig. 5 increase with increasing SEL, the relatively low slope estimates may be a result of the very small amounts of TTS observed. It is likely that the observed growth rate would increase if larger SELs (and thus larger amounts of TTS) were employed. Experiments producing larger amounts of TTS are necessary to estimate the growth rate of TTS beyond the range of the TTS amounts experimentally observed.

C. Recovery from TTS

Figure 6 summarizes the recovery from statistically significant amounts of TTS_4 observed during experiment 3. For TTS_4 of about 3–4 dB (exposure SELs of 195-199 dB re 1 μPa^2 s), recovery is nearly complete (i.e., TTS was no longer measurable) by 10 min post-exposure. For exposure SELs of 201 and 203 dB re 1 μPa^2 s, TTS_4 was larger (4–5 dB) and recovery was not complete by 10 min. The recovery curves decreased monotonically with increasing time, except for the 201 dB re 1 μPa^2 s curve; however, since the standard deviations associated with each TTS data point were 1–2 dB, this pattern should be treated with some caution. The recovery times are related to the initial TS and exposure SEL: higher SELs produced larger initial shifts which required longer recovery times. Studies of TTS in people exposed to noise also revealed that the exposure duration is important in recovery; that given identical initial shifts, longer duration exposures required longer recover times (e.g., Mills, 1983). Nachtigall *et al.* (2004) reported recovery times for slightly higher amounts of TTS (TTS_5 about 7 dB) to be much longer (up to 105 min), suggesting that this relationship may occur in dolphins as well. As with the TTS growth data, the interpretation of the recovery curves is hampered by the very small amounts of TTS relative to the variability of the measurements. Larger amount of initial NITS and longer recovery times are necessary for proper analysis. Because of the difficulties involved in generating underwater SPLs in excess of 190–195 dB re 1 μPa in a normal laboratory setting, a larger initial NITS will most likely only be accomplished by increasing exposure duration.

D. Behavioral reactions

Behavioral reactions of the subjects of experiments 1 and 2 of the present study were reported by Finneran and Schlundt (2004), who summarized behavioral reactions of dolphins and white whales exposed to 1-s tones during TTS experiments [experiments 1 and 2 of the present study and the experiments described by Schlundt *et al.* (2000)]. Observed behavioral reactions in the present study were relatively minor. The most common example of altered behavior was a reluctance or refusal to return to the S1 station on the dive immediately following the fatiguing sound exposure. On two occasions during experiments 1 and 2, NAY ignored the trainer following the exposure, preventing TTS_4 from being obtained in one instance. In general, BEN was very tolerant of the exposures and exhibited only minor changes in behavior. For experiment 3, the percentage of sessions with behavioral reactions as a function of exposure SEL is shown in Fig. 7. The small number of exposures at each SEL contributed to large variability within the dose-response data of Fig. 7 (e.g., with only two data points, all percentages will be 0, 50%, or 100%). Coupled with the lack of observed percentages greater than 50%, this prevented a true dose-response curve from being created.

E. TTS Dose-response curve

Predictions for the effects of sound on marine mammals often rely upon some numeric "threshold for effect," where sound exposures less than the threshold are assumed to cause no effect and those above the threshold are assumed to cause the effect. Ideally, a specific numeric threshold value is derived from a dose-response curve relating the sound exposure to the percentage of individuals experiencing a particular effect. A suitable value is then chosen for the percentage of individuals affected, often the 50% point, and the exposure threshold value interpolated from the dose-response curve. Similar approaches are common in pharmacological studies to determine the efficacy of drugs, studies of animal mortality in response to underwater blasts (e.g., Yelverton *et al.*, 1973), and have been used to summarize behavioral responses to noise (e.g., Finneran and Schlundt, 2004).

Figure 8 was created by combining the results of the present study with the 3-, 10-, and 20-kHz TTS data from Schlundt *et al.* (2000) to show the percentage of exposures that resulted in TTS as a function of exposure SEL. The percentage of exposures resulting in TTS is small at relatively low SELs and increases with increasing SEL, as expected. The curve resulted in a 50% affected point of approximately 206 dB re 1 μPa^2 s; however, this value should be interpreted with caution since the data are limited and the 50% point lies beyond the range of measured values. Also, because of the limited number of subjects (8), the data used to create the dose-response curve are based on the percentage of *exposures* in which TTS was observed, not the percentage of individuals experiencing TTS, and therefore much of the data come from only a few subjects. It is important to note that not all exposures above a certain TTS threshold will necessarily cause TTS; at a SEL of 195 dB re 1 μPa^2 s, the level required for significant TTS_4 in this study, the percentage of exposures which actually resulted in measurable TTS is only about 18%.

F. Summary of existing cetacean TTS data

Figure 9 summarizes the existing onset-TTS data for cetaceans. These data emphasize that the effects of the different sound exposures do not depend on the sound pressure alone, but also depend on the duration. As the exposure duration decreases, higher SPLs are required to cause TTS. In contrast, SELs required for TTS do not show the same type of variation with exposure duration, since the duration is included in the SEL calculation. The equal energy line at 195 dB re 1 μPa^2 s fits the tonal and noise data (the nonimpulsive data) well, despite differences in exposure duration, SPL, experimental techniques, and subjects. Together, these data indicate that a SEL of 195 dB re 1 μPa^2 s is a reasonable threshold to use for onset-TTS in dolphins and white whales exposed to mid-frequency sounds.

V. CONCLUSIONS

Amounts of TTS_4 measured in bottlenose dolphins after exposure to 3-kHz tones with SELs $\geq$ 195 dB re 1 μPa^2 s were significantly higher than amounts of TTS_4 measured after control sessions with simulated exposures. These data agree with prior TTS data from Schlundt *et al.*, 2000) and Nachtigall *et al.*, 2004). Together, these data point to a SEL of 195 dB re 1 μPa^2 s as a threshold for onset-TTS in dolphins and white whales exposed to midfrequency sounds. The data from the present study also suggest that the masking noise employed by Schlundt *et al.* (2000) did not have a substantial effect on the onset-TTS levels observed.

Because of the small amounts of TTS_4, estimates of the rate of growth of TTS with increasing exposure may be under-estimates. Experiments producing larger amounts of TTS will be necessary to estimate the growth rate of TTS beyond the range of the TTS amounts experimentally observed. Similarly, larger amounts of initial NITS will be required for proper measurements of recovery time.

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