Onset, growth, and recovery of in-air temporary threshold shift in a California sea lion (*Zalophus californianus*)

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(Received 23 July 2007; accepted 10 August 2007)

A California sea lion (*Zalophus californianus*) was tested in a behavioral procedure to assess noise-induced temporary threshold shift (TTS) in air. Octave band fatiguing noise was varied in both duration (1.5–50 min) and level (94–133 dB re 20 μPa) to generate a variety of equal sound exposure level conditions. Hearing thresholds were measured at the center frequency of the noise (2500 Hz) before, immediately after, and 24 h following exposure. Threshold shifts generated from 192 exposures ranged up to 30 dB. Estimates of TTS onset [159 dB re (20 μPa)^2 s] and growth (2.5 dB of TTS per dB of noise increase) were determined using an exponential function. Recovery for threshold shifts greater than 20 dB followed an 8.8 dB per log(min) linear function. Repeated testing indicated possible permanent threshold shift at the test frequency, but a later audiogram revealed no shift at this frequency or higher. Sea lions appear to be equally susceptible to noise in air and in water, provided that the noise exposure levels are referenced to absolute sound detection thresholds in both media. These data provide a framework within which to consider effects arising from more intense and/or sustained exposures.
 [DOI: 10.1121/1.2783111]

PACS number(s): 43.80.Nd, 43.80.Lb [WWA] Pages: 2916–2924

I. INTRODUCTION

Noise-induced temporary threshold shift (TTS) is the reversible elevation in hearing threshold caused by a fatiguing auditory stimulus. As a step toward understanding the potential impacts of underwater anthropogenic noise on marine life, research efforts have focused on conducting laboratory studies with the intent of examining TTS in marine mammals. Marine mammals that have been tested for susceptibility to TTS are the beluga (*Delphinapterus leucas*), bottlenose dolphin (*Tursiops truncatus*), California sea lion (*Zalophus californianus*), harbor seal (*Phoca vitulina*), and northern elephant seal (*Mirounga angustirostris*) [(Kastak and Schusterman, 1996; Kastak *et al.*, 1999; Finneran *et al.*, 2000, 2002, 2003; Nachtigall *et al.*, 2003, 2004; Finneran *et al.*, 2005; Kastak *et al.*, 2005)]. With the exception of a preliminary observation by Kastak and Schusterman (1996), none of these studies has investigated the effects of aerial noise exposure on hearing sensitivity.

Over the past several years, concerns have focused on underwater anthropogenic noise because of increases in overall level as well as correlations between cetacean (whales, dolphins, and porpoises) strandings and military operations using sonar (see, e.g., Frantzis, 1998; Fernandez *et al.*, 2005). Within marine mammals, the cetaceans are fully aquatic and therefore not considered to be susceptible to damaging effects of airborne noise; consequently, most of the literature regarding hearing loss in marine mammals has focused on underwater noise exposure. The pinnipeds (seals, sea lions, and walruses), however, represent a special case in any discussion of noise-induced TTS, because this group is amphibious and is therefore subject to the effects of noise in

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air as well as under water. Although underwater noise could disrupt foraging and predator avoidance in pinnipeds, airborne noise has the potential to disrupt social functions that involve acoustic communication, such as mother-pup bonding and breeding behavior. Furthermore, depending on the rate of recovery, hearing loss induced in air may have major impacts on auditory sensitivity under water and vice versa. Therefore, the present study was designed to examine the effects of airborne noise exposure on a California sea lion, an animal that makes frequent transitions from land to water.

The subject of this experiment had previously participated in two studies involving underwater noise exposure designed to induce small to moderate levels of recoverable hearing loss (Kastak *et al.*, 1999, 2005). These earlier experiments showed that small amounts (<10 dB) of TTS could be induced by exposure to octave bands of noise at or above 80 dB relative to threshold at the center frequency of the noise band.

Despite the results obtained in the underwater studies, there are several factors that make the noise exposure component of TTS experiments more desirable to conduct in air than in water. Under water the subject must surface in order to breathe and to obtain food reinforcement, causing variability in the received sound field. In air, only a small movement of the head is required to receive reinforcement, provided it is delivered to a precise location close to the subject's head. Further consistency in noise exposure and in threshold determination can be ensured by testing in a sound-attenuating hemi-anechoic chamber, which has the additional benefit of reducing interference from outside noises that would mask the test signal or distract the subject. Finally, in-air sessions can be conducted more rapidly than those taking place under water, permitting the rapid acquisition of the large data set needed to test for statistical significance and to obtain robust estimates of TTS onset, growth, and recovery. For these reasons, the present experiment, designed to induce TTS of 10 dB or more, was conducted in air. This level was chosen because at low noise levels (smaller threshold shifts), growth of TTS is small and therefore might be underestimated unless higher threshold shift levels (corresponding to faster growth rates) are attained.

In order to further examine the effects of noise exposure on pinniped hearing, the experimental goals were to (1) show that TTS in air is correlated with some aspect of sound magnitude; (2) determine whether TTS in air and underwater are similar when the exposure levels are related to the subject's thresholds in air and under water; and (3) assess the time course of recovery from threshold shifts induced by noise levels of up to 104 dB above the subject's threshold.

In the discussion of the exposure parameters and data obtained in this experiment, the following abbreviations are used (ANSI, 1994): SPL=sound pressure level (dB re 1 μPa in water and dB re 20 μPa in air); SL=sensation level, or the difference between noise SPL and the subject's auditory threshold (dB); SEL=sound exposure level [dB re 1 μPa^2 s in water and dB re $(20\ \mu Pa)^2$ s in air]. Sound exposure level was used to quantify the fatiguing stimulus so that both sound magnitude and temporal variables could be taken into account simultaneously.

II. MATERIALS AND METHODS

A. Subject

The experimental subject was a female California sea lion (*Zalophus californianus*) named "Rio," whose age ranged from 17 to 20 years during the course of the experiment. She was housed at Long Marine Laboratory in Santa Cruz, CA in pools filled with free-flowing seawater surrounded by haulout space. She was maintained on a fish diet comprising herring and capelin, and received approximately 50%–75% of her daily ration during experimental sessions.

Rio had extensive prior experience in behavioral psychoacoustical testing, including underwater and aerial audiometry (Kastak and Schusterman, 1998; Southall *et al.*, 2005), auditory masking (Southall *et al.*, 2003, 2005), in-air sound localization (Holt *et al.*, 2005), and underwater noise-induced TTS (Kastak *et al.*, 1999, 2005). Prior training in these tasks facilitated acquisition of the behaviors involved in the current study. The protocols used in all stages of training and testing were approved by the UCSC Chancellor's Animal Research Committee. Research was conducted under NMFS Permit Nos. 259-1481-00 and 1072-1771-00.

B. Apparatus

Testing was conducted in a hemi-anechoic chamber (Eckel Industries), located next to the subject's living space. The chamber was a 2.5 m tall rectangular structure that was divided into a 3×5.6 m experimental space where the subject was tested, and a 3×1.4 m control room where an experimenter, an assistant, and the controlling equipment were located. The experimental space and control room were double-walled to eliminate environmental noise contamination. The walls and ceiling of the experimental space were lined with fiberglass-filled stainless steel wedges, and the concrete floor was covered with 2.6-cm-thick closed-cell neoprene. These features provided a relatively uniform acoustic space. Ventilation was provided by quieted fans that were not acoustically coupled to the chamber.

Inside the experimental space, a threshold station, response target, two speakers, and trial light were used for audiometric testing. The threshold station was a 33 cm tall PVC stand atop of which a PVC chin cup was mounted. The 10×10 cm response paddle was mounted 50 cm to the left of the threshold station at the level of the chin cup. The test tone projector, located on-axis to the chin cup at a distance of 70 cm, was a JBL 2123H midrange speaker. The trial-indicator light was placed just below this speaker, and was used to denote the interval of each experimental trial. The second speaker was placed on the floor of the chamber next to a PVC tube that was used to deliver fish reinforcement to the subject. The second speaker emitted an acoustic tone (bridge stimulus) to signal the subject that fish would be delivered.

To the right of the threshold station, a noise exposure station, a noise-projecting speaker, and a station-indicator light were configured for use during noise exposures. The noise exposure station was identical to the threshold station but placed 60 cm to its right and at an angle of approximately 45° to the right. This station faced the noise exposure speaker that was centered 35 cm in front of it. The noise exposure speaker was either a Fender Princeton Chorus Guitar Amplifier or a Community EM280 compression driver coupled to a P100 horn projector. The light mounted just above the noise exposure speaker was used to cue the subject to remain positioned at the noise generating speaker until reinforcement was provided. During exposure, reinforcement was delivered from the same location as in threshold testing.

C. Acoustic stimuli and measurement

In order to provide continuity from prior underwater testing, pure tone thresholds were determined using a 2.5 kHz stimulus and noise exposures comprised an octave band centered at 2.5 kHz. The center frequency of the noise band was chosen in favor of a frequency $\frac{1}{2}$ octave above the center frequency because previous data from the same subject showed lack of a $\frac{1}{2}$ octave effect (Kastak *et al.*, 2005). Audiometric testing was semiautomated, using LABVIEW (National Instruments) virtual instruments. Pure-tone signals were converted from digital to analog at an update rate of 32 kHz using a National Instruments PXI-6070 multifunction DAQ device housed in a PXI 8176 controller. The pure-tone signals were 500 ms in duration with 40 ms linear rise/fall times and were projected from the JBL 2123H speaker. Octave-band noise with a center frequency of 2.5 kHz was used for noise exposure. This fatiguing noise was generated on the PXI board, digitally filtered, and bandpass filtered using a Krohn-Hite 3530 filter in order to obtain a flat frequency spectrum. It was amplified using a Hafler P9000 power amplifier, and projected from the Fender amplifier during the first phase of the experiment and the Community projector during the second phase.

Signals and noise were calibrated in the acoustic chamber using a Josephson Engineering C550H microphone and either a PC-based signal analysis package (Spectra Plus, Pioneer Hill) or a combination of virtual instruments. Mapping of the sound field in 10 cm^2 grids surrounding the stations ensured that spatial variability of the acoustic stimuli was less than ±2 dB in the vicinity of the subject's head.

D. General procedure

TTS was measured as a function of noise SL, SPL, SEL, and duration. The experimental design called for holding the frequency constant over experimental sessions while varying the level and duration of the fatiguing noise. Measurement of TTS was accomplished by assessing the subject's hearing sensitivity to the test tones before, immediately after, and at least 24 h following exposure to the octave-band noise. Control sessions, which comprised threshold testing associated with mock noise exposure, were also conducted. Multiple replicates of each exposure and control condition were run to ensure that statistically reliable results would be obtained. There were two phases of the experiment, and each phase followed an exposure matrix which defined the levels, durations, and number of replicates to be used. The sequence of testing was pseudorandomized within each phase of testing.

The subject's performance in all stages of testing was voluntary, with behavioral control established by operant conditioning and food reinforcement. During testing the subject was free to terminate participation in the experiment at any time by moving to the door of the acoustic chamber. When this occurred, the experiment was stopped and she was allowed to return to her pool enclosure. This happened rarely and primarily during initial training. There was no relation between the level of the noise and self-termination of sessions.

1. Preexposure threshold determination

A modified staircase method and a go/no-go procedure were used to estimate absolute hearing thresholds. Based on previous audiometric data, a test signal with a level of approximately 25 dB SL served as the stimulus for the first signal trial. A trial began with the subject positioned at the threshold station. The light situated under the tone projection speaker was turned on by the experimenter to initiate a trial. Following a brief, randomized delay of 1–4 s, a pure-tone signal was projected. The subject responded to the presence of the signal by touching the left-mounted response paddle with her nose (HIT). When this occurred, the bridge stimulus was projected and she received a fish delivered through the PVC tube by an assistant in the control room. The ratio of signal trials to no-signal trials (catch trials) was 1:1. Withholding response on a catch trial (CORRECT REJECTION) was reinforced in the same way as a HIT (bridge followed by food reinforcement). There were two types of incorrect response: failing to touch the paddle on a signal trial (MISS) and touching the paddle on a catch trial (FALSE ALARM). The sea lion received no special feedback following either type of incorrect response, except that the trial light was turned off. In these situations the subject normally restationed and waited for the next trial to begin.

On the first signal trial of a session, the maximum tone level (25 dB SL) was presented. Following each HIT, the level was dropped by 4 dB. This process continued until a MISS occurred. Following each MISS the level of the signal was raised by 2 dB. The 2 dB level change was used on all subsequent signal presentations (lowered following each HIT and raised following each MISS). Following nine reversals (transitions from HIT to MISS or vice versa), the signal level was raised to the starting level for several cooldown trials designed to maintain stimulus control over the response behavior. Thresholds were determined following the method of Dixon and Mood (1948) after the conclusion of the session. Data from sessions in which the false alarm proportion exceeded 0.25 were discarded. After the hearing threshold was determined, the subject was given a 20–30 min break prior to beginning the noise exposure.

2. Noise exposure

Noise exposure began when the subject was cued to enter the acoustic chamber and position at the exposure station. The exposure noise was turned on prior to the subject entering the chamber. An octave band of Gaussian white noise was projected from the noise-projecting speakers with a level and duration chosen from a predetermined exposure matrix. Approximately once every 30 s during noise exposure the

TABLE I. Noise exposure matrices for Phase 1 and Phase 2. The noise levels used are expressed in dB SL and dB SPL. The number of sessions conducted for the 16 level/duration combinations are provided, yielding a total of 112 exposure sequences for Phase 1 and 108 exposure sequences for Phase 2. The sound exposure levels resulting from each level/duration combination are shown in the cells.

Phase 1						
Noise exposure			Sound exposure level			
dB SL	dB SPL	*n*	1.5 min	12 min	25 min	50 min
Control	⋯	4	⋯	⋯	⋯	⋯
65	94	8	113	122	125	128
80	109	8	128	137	140	143
95	124	8	143	152	155	158

Phase 2						
Noise exposure			Sound exposure level			
dB SL	dB SPL	*n*	6.25 min	12.5 min	25 min	50 min
Control	⋯	3	⋯	⋯	⋯	⋯
98	127	8	152	155	158	161
101	130	8	155	158	161	164
104	133	8	158	161	164	167

light was turned off and a fish was delivered to the subject. At these times, the subject's position within the noise field changed only briefly when she moved from the station in order to pick up her fish. The time spent out of the calibrated noise field was small (on the order of several seconds) compared to the overall time of exposure; therefore, only energetically negligible differences in actual versus estimated exposure levels occurred. When the full duration of exposure was achieved, the light and noise at the exposure station were turned off. The sea lion was rewarded with a piece of fish and cued to position at the threshold station where postexposure testing immediately began.

3. Postexposure threshold testing

Following cessation of the noise exposure interval, the sea lion's hearing threshold to the 2.5 kHz test tone was assessed again. Actual determination of the threshold took place between 10 and 15 min following cessation of noise exposure. TTS was measured as the difference in decibels between postexposure and preexposure thresholds. The subject's hearing was tested again the following morning in order to assess whether sensitivity had fully recovered. In all cases where the initial postexposure threshold was more than 20 dB higher than the corresponding preexposure threshold, the subject was retested later the same day. In cases where the retest threshold was still elevated by 3 or more dB from the preexposure threshold, the subject was retested on subsequent days. Testing continued without intervening noise exposures until the threshold was within ±3 dB of the baseline threshold.

E. Exposure conditions

The exposure matrices used in Phase 1 and Phase 2 of the experiment are shown in Table I, which provides the SPL and the SEL for each condition tested. Each exposure matrix was a 4×4 design, with four exposure levels (three noise levels plus the control condition) and four exposure durations. Eight replicates of each of the noise exposure cells and three to four replicates of each of the control exposure cells were completed in a pseudorandomized order within each matrix.

The noise exposure levels in each matrix were established relative to the subject's baseline auditory threshold at 2.5 kHz. The subject's mean threshold at 2.5 kHz was 29 dB SPL prior to the start of the experiment, and all exposures levels were selected relative to this threshold. Therefore, an exposure level of 95 dB SL corresponded to an absolute exposure level of 29+95, or 124 dB SPL.

Phase 1 of the experiment took place between July 2002 and May 2003. In this phase, the exposure levels were 65, 80, and 95 dB SL. The exposure durations corresponding to each of these levels were 1.5, 12, 25, and 50 min.

Phase 2 of the experiment took place between April 2005 and October 2005. The exposure levels were 98, 101, and 104 dB SL and the exposure durations were 6.25, 12.5, 25 and 50 min. These exposure levels were incremented in 3 dB steps and the durations were incremented by doubling. This matrix was designed so that sound levels and durations increased in a systematic manner, both to induce larger shifts, and to allow for further comparison of equal SEL conditions comprising different combinations of exposure SPL and duration.

F. Analysis

Threshold shifts were calculated by subtracting preexposure thresholds from postexposure thresholds. Mean threshold shifts paired by sequence were compared across preexposure, postexposure, and 24 h recovery conditions using repeated-measures ANOVA, followed by a Student-Neuman-Keuls procedure for individual comparisons in the event that the ANOVA results were significant at the 0.05 level. Mean thresholds obtained using various combinations of duration and SPL resulting in equal sound exposure were also compared, using a Student's t-test or one-way ANOVA. Given

TABLE II. The mean TTS values obtained for each cell of the noise exposure matrices used in Phases 1 and 2. Statistically significant threshold shifts are indicated as $^{*}(p<0.05)$, $^{**}(p<0.01)$, and $^{***}(p<0.001)$. Note that none of the control (no-noise) conditions generated significant TTS.

Phase 1						
Noise exposure			Mean TTS			
dB SL	dB SPL	n	1.5 min	12 min	25 min	50 min
Control	⋯	4	0.7	1.1	0.7	−1.4
65	94	8	0.0	2.2	4.7**	1.0
80	109	8	−0.5	4.5**	5.3***	7.2***
95	124	8	1.5	3.8**	6.1***	11.0***
Regression slope			0.05	0.06	0.05	0.34***

Phase 2						
Noise exposure			Mean TTS			
dB SL	dB SPL	n	6.25 min	12.5 min	25 min	50 min
Control	⋯	3	0.5	−0.1	1.4	−0.7
98	127	8	1.7*	4.3***	7.3***	10.5***
101	130	8	3.3**	5.7***	7.8***	16.0***
104	133	8	4.6***	7.6***	12.3***	23.5***
Regression slope			0.50**	0.55**	0.84*	2.12***

that the difference in SEL between the 12 and 12.5 min exposures used in Phases I and II is negligible, the data from these two conditions were combined. Threshold shifts across equal sensation levels in air (this study) and under water (Kastak *et al.*, 2005) were also compared using a Student's t-test. A two-way ANOVA was used to test the effects of noise level and duration on the magnitude of TTS, as well as to test for interaction between the two factors.

Where SELs were plotted against threshold shifts, the following equation was used to fit to the data:

$$\mathrm{TTS} = (10m1)\log_{10}(1 + 10^{(\mathrm{SEL}\text{-}m2/10)})$$

(Kastak *et al.*, 2005). This is a modified form of the equation used to fit asymptotic threshold shift used by Maslen (1981). The parameters of the equation refer to TTS onset ($m2$) and growth of TTS with increasing SEL ($m1$). The latter parameter corresponds to the slope of a straight line fitted to the linear portion of the curve while the former corresponds to the x intercept (threshold shift of zero) of the same line. This equation is descriptive, and is not necessarily explanatory of the relationship between TTS and SEL.

III. RESULTS

Threshold shifts obtained for each exposure condition are shown in Table II. Significant differences between preexposure and postexposure thresholds are marked with asterisks. There were no differences between mean preexposure and 24 h recovery thresholds under any combination of level and duration (with the exception of six threshold values that reflected longer-duration recovery, obtained at intervals greater than 24 h). In Phase I, threshold shifts were not observed at durations of 1.5 min regardless of noise SPL. The only significant linear trend toward increasing threshold shifts with increasing stimulus level occurred at an exposure duration of 50 min, and the slope was extremely small (0.3 dB/dB noise SPL). In Phase II, significant threshold shifts were detected at all levels tested. Mean shifts ranged from 1.7 dB at 98 dB SL/6.25 min to 23.4 dB at 104 dB SL/50 min. All relations between threshold shift and SPL were significant, with small (less than 1) but positive slopes under all durations except the 50 min condition, in which the slope was 2.2 dB TTS/dB noise SPL.

There were no threshold shifts on any control sequence, indicating that no factor other than noise was responsible for the temporary loss of hearing sensitivity observed in the present study. No trends in preexposure thresholds were observed within each phase; however, mean baseline thresholds increased slightly (by 1.5 dB) between the phases (approximately one year). This difference was significant at the 0.5 level, implying some hearing loss between the two phases. However, an audiogram obtained from this subject several months after completion of the experiment showed no significant threshold shift at the test frequency relative to the mean of the Phase 1 preexposure thresholds. These results are difficult to interpret, although the audiogram did show some loss in sensitivity at frequencies above 6.4 kHz. Whether this increase is noise induced or age related cannot be determined; however, the phenomenon of high-frequency hearing loss in the absence of controlled noise exposure has been observed in another individual of the same species (Schusterman *et al.*, 2002).

Results of the 25 and 50 min exposures at 95 dB SL, when directly compared to results obtained under water under the same exposure conditions, revealed no significant differences in mean threshold shifts between the two media, as shown in Fig. 1. An additional comparison between underwater exposures at 80 dB SL and 22 min duration and in-air exposures at the same sensation level at 25 min also indicated no significant differences between the two media.

Comparisons of threshold shifts among equal-energy (SEL) conditions comprising various combinations of level and duration showed a trend toward increasing threshold

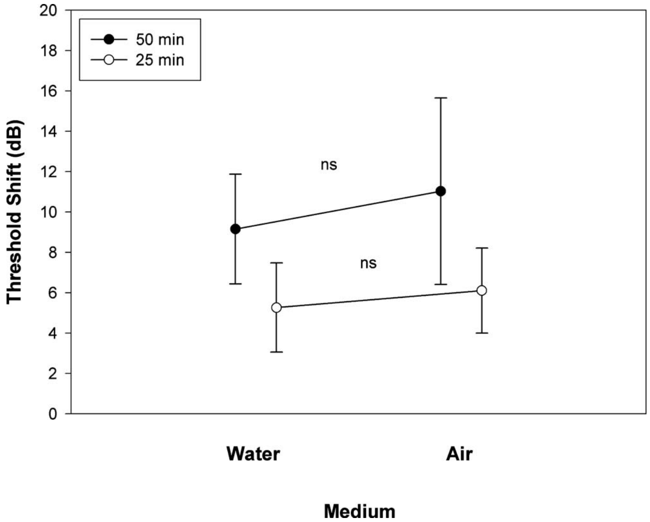

FIG. 1. Comparison of mean threshold shifts induced by equivalent SL exposures (95 dB SL) in air and under water for two duration conditions. There were no significant differences attributable to the differences in media.

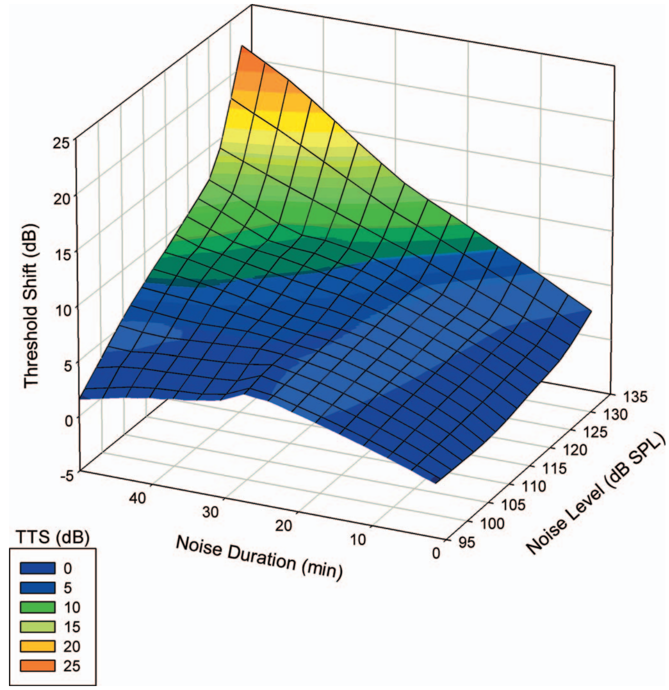

FIG. 2. A surface plot fitted to the mean threshold shifts under all conditions of noise level and duration. There is a significant interaction between noise SPL and duration for both phases of the experiment.

shifts with increases in duration (Table III). This effect was most profound at higher sound exposure levels. In equal energy comparisons, longer duration exposures always resulted in greater mean threshold shifts than louder, shorter durations of the same overall sound energy.

Figure 2 shows a response surface fitted to a three-dimensional plot of noise duration and SPL versus threshold shift, with the data points removed for clarity. The effect of increasing duration can be seen to be largest at higher sound pressure levels (i.e., the duration effect depends on the level of noise), reinforcing the effect shown in Table III. Threshold shifts increased slowly with increasing duration at the lowest noise exposure levels, and increased rapidly with duration at the highest noise exposure levels used. The interaction between sound pressure level and duration was statistically significant for both Phase I ($F_{6,84}=5.28$, $p<0.001$) and for Phase II ($F_{6,84}=3.35$, $p<0.01$).

Figure 3 shows threshold shifts plotted against SEL. There was a significant increasing trend as shown by the experimental model, with greater SELs resulting in greater threshold shifts ($m1=2.5$; $m2=159$; $F(_{1,191})=224$; $p<0.0001$; $R^2=0.54$). The resultant level of TTS onset was thus determined to be 159 re $(20\ \mu\text{Pa})^2$ s, while the growth of TTS at SELs above this level was 2.5 dB per dB increase in SEL. Caution must be used in the interpretation of this trend, as a relation between SEL and TTS assumes that the equal-energy rule applies, an assumption which is probably not strictly correct, as shown by the equal SEL comparisons.

In six exposure sequences, threshold shifts exceeded 20 dB. In these cases, repeated threshold estimates were obtained at postexposure intervals ranging from 1 to 48 h. Recovery functions for these sequences are shown in Fig. 4. All thresholds eventually returned to acceptable baseline levels (26–32 dB SPL), and all recovery functions were similarly shaped. A regression of threshold shifts versus the logarithm of time showed a highly significant linear relation, with threshold shifts decreasing at a rate of 8.8 dB per log (min).

TABLE III. Temporary threshold shifts obtained at combinations of noise SL/duration. Equal sound exposure level conditions are grouped.

Sound exposure level (SEL)	Level/duration combination (dB SL/min) resulting in column 1 SEL	TTS (note increasing TTS with increasing duration)
128	80 dB SL/1.5 min	−0.5
	65 dB SL/50 min	1.0
143	95 dB SL/1.5 min	1.5
	80 dB SL/50 min	7.2
152	98 dB SL/6.25 min	1.7
	95 dB SL/12 min	3.8
155	101 dB SL/6.25 min	3.3
	98 dB SL/12.5 min	4.3
	95 dB SL/25 min	6.1
158	104 dB SL/6.25 min	4.6
	101 dB SL/12.5 min	5.7
	98 dB SL/25 min	7.3
	95 dB SL/50 min	11
161	104 dB SL/12.5 min	4.6
	101 dB SL/25 min	7.8
	98 dB SL/50 min	10.5
164	104 dB SL/25 min	12.3
	101 dB SL/50 min	16

IV. DISCUSSION

The experiment described here represents the most robust and conclusive data set on TTS in a marine mammal to

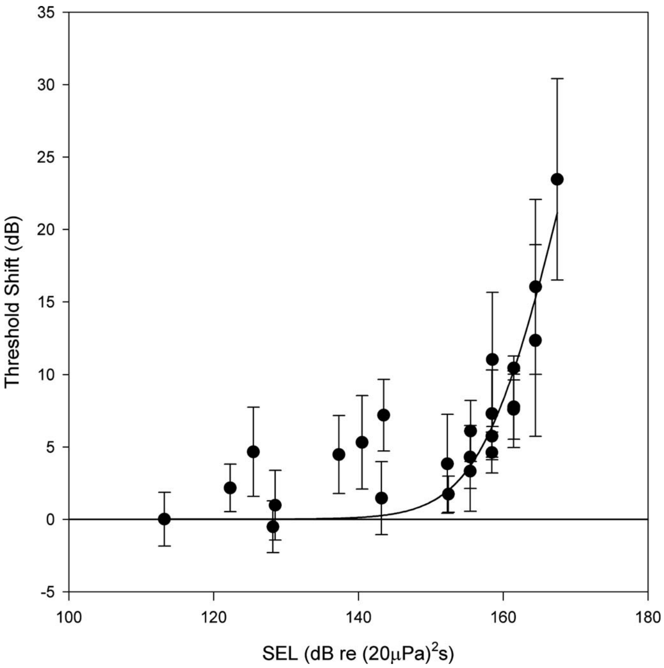

FIG. 3. A plot of TTS in dB vs SEL in dB re (20 μPa)^2 s, showing an exponential increase in magnitude of TTS with increasing exposure levels. The symbols represent mean shifts and the error bars represent standard deviations.

date. Onset, growth, and long-term recovery from TTS were statistically characterized with the benefit of the large data set that could have only been obtained under testing situations similar to those outlined here. Previous studies (Kastak *et al.*, 1999, 2005) have suffered from a limitation in the amount of data that could be collected, primarily because testing occurred in water. Variability of results in the previous studies meant that either a linear model or a nonlinear model with fixed parameters had to be used to fit the data. A linear model presented an unrealistically low TTS onset as well as a shallow slope that most likely underestimated the growth of TTS considerably (Kastak *et al.*, 1999). A curvilinear model based on a small data set with relatively small TTS values required at least one of the model parameters (slope) to be fixed, reducing confidence in the model (Kastak *et al.*, 2005). In the present experiment, both model parameters (slope and onset) were permitted to vary without bounds, resulting in a highly significant fit of the experimental model. This was likely due to the large number of data points as well as the wide range of TTS values included. Overall, the data presented here provide a clearer description of TTS in this species than has previously been the case.

It is important to note, however, that this study has limitations. First, the noise exposure was chosen with very specific characteristics (center frequency and bandwidth), as was the audiometric test stimulus. It is possible that different results would have been obtained had the experimental parameters varied along these dimensions. Second, only one subject was used, a deficiency that is, unfortunately, common in experimental work with marine mammals. However, these factors should not minimize the importance of the results; the subject was repeatedly tested to generate a robust assessment of onset, growth, and recovery of aerial TTS.

Growth of TTS, estimated here as 2.5 dB/dB increase in noise level, is a particularly important piece of data in the assessment of noise-induced hearing loss. It is difficult to quantify, however, and therefore hearing loss at nontested SELs is difficult to predict. At low to moderate SELs, a linear fit best describes the data; however, based on such an analysis, the growth of TTS is unrealistically small (only a fraction of a decibel increase in TTS per decibel increase in noise level). Further, linear models indicate a progressive threshold improvement as noise levels decrease, sometimes intercepting the abscissa at levels that would lead to unreasonably low noise levels corresponding to TTS onset. Rather, a model describing TTS growth should be curvilinear and have a lower asymptote at 0 dB TTS. The data we obtained are better fit by an exponential equation, the parameters of which correspond to TTS onset and growth at moderate to high levels of noise (Maslen, 1981; Kastak *et al.*, 2005). For this subject in air, TTS onset was established at 159 dB SEL [dB re (20 μPa)^2 s]. Growth of TTS was determined to be slightly above 2 dB per dB increase in exposure level. Both of these figures can be reasonably applied to noise exposure in California sea lions in air, with the following caution: Sound exposures that are different enough to be considered qualitatively different (impulse versus continuous, noise versus tone, etc.) are not likely to obey simple rules for predicting noise impacts.

A potential pitfall in using a SEL approach to quantifying TTS was made evident in an analysis of the 18 equal energy exposure conditions presented in this study. In every

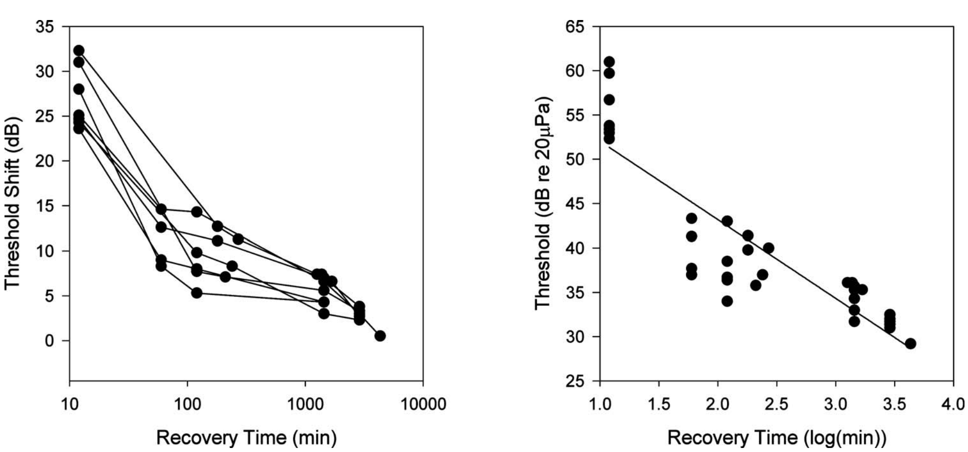

FIG. 4. Recovery from TTS greater than 20 dB plotted against the logarithm of time. All shifts eventually returned to near zero. Each line represents an individual test-recovery sequence. (b) A linear regression applied to the pooled data. The slope of the line is significant, $p < 0.001$, with a slope of −8.8 dB per log(min). The R^2 value is 0.54.

sequence comprising equal energy exposures, the longer, quieter exposure resulted in a greater threshold shift than the shorter louder exposure, which was an unexpected result (Ward, 1962). These results cannot be overlooked when making predictions of the sort that rely on an energy-based relationship. It is possible that peculiar anatomical properties, balances between simultaneous hearing loss and recovery, or differing physiological effects of noise play a significant role in governing the amount of damage a particular noise exposure may cause. Because the results of equal SEL comparisons shown in Table III appear to contradict the equal energy trading rule, a more productive way to examine the TTS data presented here may be to examine the relationship among SPL, duration of exposure, and the magnitude of TTS. The interaction between SPL and duration shown in Fig. 2 confirms that neither factor can be used alone to predict the magnitude of TTS. However, such a graph shown may provide an extremely powerful tool for predicting the potentially detrimental effects of noise on the hearing of a particular species of interest. For the California sea lion in this study, an exposure of 130 dB SPL for a duration of 20 min might be expected to induce a moderate threshold shift on the order of 5 dB, while an exposure of 120 dB lasting 40 min might induce a more severe shift of 10–15 dB. This example demonstrates the disproportionate effect of duration over SPL on the magnitude of TTS induced by noise. It further illustrates the usefulness of such a graph in predicting anthropogenic effects on hearing in free-ranging marine mammals, given that received levels are known or can be estimated. The shapes of these response surfaces will likely vary with sound type and frequency range, as well as species of interest, but the approach remains valid nonetheless.

Noise exposure levels used in this study and in previous research on pinniped TTS (Kastak *et al.*, 1999, 2005) were chosen by referencing them to the subject's threshold at center frequency, expressed as dB SL (sensation level). The use of SL to normalize exposure levels for comparisons across individuals and species is unproven and results have been equivocal. This approach is likely to be valid across the subject's area of best sensitivity—obtaining equal loudness curves for these species would be a good first step in determining noise effects outside areas of best sensitivity. Subjects with higher baseline hearing levels have been shown to require proportionally higher noise exposures to induce threshold shifts in other mammals (Humes and Jesteadt, 1991; Boettcher, 2002), and an approach based on SL was successful when harbor seal and sea lion results were compared (Kastak *et al.*, 2005); however, results obtained from a northern elephant seal were contradictory.

Sensation level may also be a useful tool for comparing the effect of noise SEL between air and water. For example, TTS onset under water for this subject was about 206 dB re 1 $\mu\mathrm{Pa}^2$ s (Kastak *et al.*, 2005), which is equivalent to noise exposure at a level of 206 dB re 1 μPa lasting 1 s. This SPL is 131 dB greater than the subject's absolute underwater threshold (i.e., 131 dB SL). In air, TTS onset for the same stimuli was estimated as 159 dB re $(20\ \mu\mathrm{Pa})^2$ s, corresponding to noise exposure of 159 dB re 20 μPa lasting 1 s, or an onset level of 130 dB SL. Thus, comparisons of the present data with data previously obtained indicate that noise exposures of equal durations can be equated in terms of SL, irrespective of medium. Because of more efficient testing procedures in air, we believe that aerial data can be used as an alternative for predicting the effects of underwater exposures to bands of noise. Tests to determine whether the same relationships also apply for different types of noise or to different aquatic species remain to be conducted.

A final, unexpected benefit of this study was the assessment of long-term recovery from TTS. Typically, recovery from TTS takes place in two qualitatively (if not physiologically) separate phases (Ward *et al.*, 1959). The first is a short-term process during which the level of TTS "bounces" to its highest level at approximately 2 min postexposure then begins to drop (Spieth and Trittipoe, 1958). Evaluation of threshold shifts during this period requires rapid audiometric methodology. The second phase of recovery is longer term, with threshold shifts generally declining in proportion to the logarithm of time (Ward *et al.*, 1959). Unfortunately, the limitations of this study resulted in postexposure threshold estimates that occurred at an average of 12 min following exposure. Whether the 2 min bounce occurs for TTS in pinnipeds is a matter reserved for future studies, but it is likely that many of the threshold shifts obtained in this study are underestimates of the maximum shift occurring at the 2 min postexposure point. It is clear, however, based on trial-to-trial data, that recovery from TTS occurs rapidly in marine mammals (on the order of minutes) for low and moderate levels of noise exposure (Finneran *et al.*, 2005; Kastak *et al.*, 2005; Nachtigall *et al.*, 2004), with TTS levels likely approaching zero within 1 or 2 h.

For threshold shifts greater than about 20 dB, the time course of TTS recovery was much longer, with a maximum of over 48 h elapsing prior to complete recovery. Recovery was proportional to the logarithm of recovery duration. Because recovery from TTS may take a considerable amount of time, it is of great importance for regulatory applications of TTS data to consider that some effects of anthropogenic noise may persist significantly beyond the duration of the noise itself. Based on the rates of recovery reported in this study, recovery from TTS appears to depend on the degree of threshold shift rather than solely on the absolute levels of noise exposure. Therefore, when considering the effects of noise on marine mammals, it is important to note that not only may a given noise exposure result in hearing loss in some species but not others, but that recovery from hearing loss will also differ among species and even individuals. Differences in susceptibility will likely be based on factors such as age, sex, and prior exposure over time scales of months or years.

Although the interpretations we make may not represent susceptibility to noise by California sea lions in general, it is clear that, for this individual, moderate and high levels and durations of broadband noise can cause auditory fatigue greater than the threshold shifts that have previously been reported for marine mammals. Based on the relation between sound exposure levels and TTS, the following may clarify what the experimental data mean in a practical sense. In water, a passing ship exposing a pinniped to noise for a period of 10–20 min would need to produce received levels of between 170 and 175 dB re 1 μPa in order to begin to induce TTS. Similarly, in air, an aircraft flying over a sea lion rookery and exposing the animals to broadband noise for 30 s to 1 min would need to generate received levels of about 140–145 dB re 20 μPa in order to induce TTS. It cannot be emphasized enough that these are highly specific and simplified examples, ignoring other effects such as startle responses, masking, and differing rates of recovery. Further, noise related effects other than TTS, such as temporary loudness shift, can affect broader frequency ranges and take much longer to recover (Botte and Monikheim, 1994). The caveats regarding sample size, noise type, and exposure intermittence remain applicable; thus, additional research must examine the effects of different noise types on this and other species. However, predictions made by this and other studies do serve to indicate potential damaging effects to the California sea lion's auditory system by relatively intense, sustained, and/or chronic anthropogenic noise exposure.

ACKNOWLEDGMENTS

The authors would like to thank the student assistants and volunteers at Long Marine Laboratory for their dedicated and hard work resulting in the completion of this project. Financial support was provided by Grant No. N00014-04-0284 from the Office of Naval Research; we thank Mardi Hastings and Robert Gisiner at ONR for their continued support. Funding for the hemi-anechoic chamber and control equipment was provided by a Defense University Research Instrumentation Program (DURIP) grant. Two anonymous reviewers provided extremely thorough and valuable comments.

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