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RESEARCH ARTICLE

Broadband noise exposure does not affect hearing sensitivity in big brown bats (*Eptesicus fuscus*)

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ABSTRACT

In many vertebrates, exposure to intense sounds under certain stimulus conditions can induce temporary threshold shifts that reduce hearing sensitivity. Susceptibility to these hearing losses may reflect the relatively quiet environments in which most of these species have evolved. Echolocating big brown bats (*Eptesicus fuscus*) live in extremely intense acoustic environments in which they navigate and forage successfully, both alone and in company with other bats. We hypothesized that bats may have evolved a mechanism to minimize noise-induced hearing losses that otherwise could impair natural echolocation behaviors. The hearing sensitivity of seven big brown bats was measured in active echolocation and passive hearing tasks, before and after exposure to broadband noise spanning their audiometric range (10–100 kHz, 116 dB SPL re. 20 µPa rms, 1 h duration; sound exposure level 152 dB). Detection thresholds measured 20 min, 2 h or 24 h after exposure did not vary significantly from pre-exposure thresholds or from thresholds in control (sham exposure) conditions. These results suggest that big brown bats may be less susceptible to temporary threshold shifts than are other terrestrial mammals after exposure to similarly intense broadband sounds. These experiments provide fertile ground for future research on possible mechanisms employed by echolocating bats to minimize hearing losses while orienting effectively in noisy biological soundscapes.

INTRODUCTION

Exposure to intense sounds seriously impacts the structural integrity and functioning of the vertebrate auditory system (Gold and Bajo, 2014). Short duration (several minutes to several hours), intense [90–120 dB sound pressure level (SPL) re. 20 µPa root mean square (rms)] exposures produce temporary threshold shifts (TTS) in hearing sensitivity that resolve over time. Cumulative short duration exposures or long duration exposures of days to weeks produce permanent threshold shifts from which recovery does not fully occur (Clark, 1991). These impairments have been documented in many vertebrate species, including fishes (Amoser and Ladich, 2003; Smith et al., 2004), birds (Ryals et al., 1999), rodents (Ryan and Bone, 1978; Boettcher, 1993; Heffner et al., 2008), marine mammals (Popov et al., 2013; Finneran, 2015) and humans (Ward et al., 1958; Mills et al., 1981). In spite of species and individual differences in the most effective acoustic parameters for producing TTS, these animals all demonstrate some hearing losses under some exposure conditions.

Comparative analyses (Smith et al., 2004) suggest that mammals are more susceptible to TTS than are fishes or birds. Among mammals, however, differences in the magnitude of threshold shifts after noise trauma have been observed, even under similar exposure conditions. Some inbred mouse (*Mus musculus*) strains (129/SvEv, Yoshida et al., 2000; MOLF/Ei, Candreia et al., 2004) appear to be more resistant to TTS than more common, wild-type (CBA) strains. Mongolian gerbils (*Meriones unguiculatis*) suffer considerable TTS at some tone frequencies, but the magnitude of the threshold shift diminishes, rather than cumulates, after multiple exposures (Boettcher, 1993). These data suggest that some resistance to noise damage may exist in some mammals, but understanding of these differences and their underlying mechanisms remains limited. Considering the variety of biotic and abiotic soundscapes in which different animal species have evolved, we here propose a novel hypothesis to understand species differences in noise susceptibility. Rodents, which are common models for TTS, have evolved in quiet natural environments and may thus be particularly susceptible to the impact of intense noise. Echolocating bats, in contrast, have evolved in noisy environments, where they are naturally exposed to continuous intense sound levels from their own and neighboring sonar emissions while foraging, orienting, and emerging from their roosts (Jakobsen et al., 2013). For bats, exposure to prolonged, intense wideband sound is an occupational hazard. Given these evolutionary pressures, we propose that bats may have developed lessened susceptibility to noise-induced hearing losses that might otherwise harm their echolocation abilities.

The big brown bat, *Eptesicus fuscus* (Palisot de Beauvois 1796), emits intense (100–120 dB SPL), broadband downward-sweeping (100 kHz to 20 kHz) frequency modulated (FM) biosonar pulses while navigating and locating prey (Surlykke and Moss, 2000; Simmons et al., 2001). To perform these tasks, bats emit rapid trains of these intense pulses and listen for returning echoes from prey and from obstacles such as trees or buildings. Echoes vary in strength from about 0 to 80 dB SPL, depending on the size of and distance to the reflecting object (Kick, 1982; Stilz and Schnitzler, 2012). To identify and classify objects, bats compare spectral and temporal characteristics of each returning echo with its preceding emission, a process involving considerable perceptual and attentional demands even in single-bat foraging events (Simmons, 2014). Bats are social animals. In their natural environments, they fly and forage in close proximity with other bats, and thus are exposed not only to their own sonar pulses and echoes but also to emissions from other echolocating bats and echoes from multiple reflecting objects (Warnecke et al., 2015). In these highly acoustically cluttered conditions, sound exposures can be essentially continuous, reaching levels of 110 to 140 dB SPL in some species (Simmons et al., 2001,

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2004; Jakobsen et al., 2013). Additionally, many species reside in crowded, noisy roosts, with some colonies housing as many as one million animals. Aggregate SPLs of echolocation calls emitted by bats emerging from these roosts can be extremely high (Simmons et al., 1978).

The intense, repeated exposures to broadband sounds experienced by bats in their natural environments might be expected to compromise the auditory capabilities essential for successful hearing and echolocation. Nevertheless, bats forage and navigate in groups quite successfully (Simmons et al., 2001, 2004; Moss and Surlykke, 2010). There is considerable interest in determining the mechanisms by which bats can produce intense sonar emissions in noisy soundscapes and yet remain sensitive to less intense echoes returning after short time delays (Nachtigall and Schuller, 2014). Some adaptations for optimal echolocation based on single- or paired-bat performance have been identified (Moss and Surlykke, 2010; Bates et al., 2011; Warnecke et al., 2015), but it is not clear how these strategies might operate to minimize the impact of repeated sound exposures on hearing ability in acoustically crowded natural environments.

Previously, we reported that auditory brainstem responses (ABR) to pure tones in another FM echolocating bat, the Japanese house bat (*Pipistrellus abramus*), did not show threshold shifts immediately or 30 min after exposure to broadband noise [sound exposure level (SEL) of 122 dB; Simmons et al., 2015]. To extend these results, we conducted psychophysical experiments that measured hearing thresholds in big brown bats to FM echoes both before and after exposure to broadband noise. The frequency composition and amplitude of the noise exposure stimulus were within the range bats encounter naturally. We hypothesized that, under the stimulus parameters used in this experiment, bats would not experience significant loss of hearing sensitivity to these FM stimuli after intense noise exposure.

MATERIALS AND METHODS
Animals
Seven adult big brown bats (four females and three males, body mass 15–19 g) were wild-caught from buildings in Rhode Island, as authorize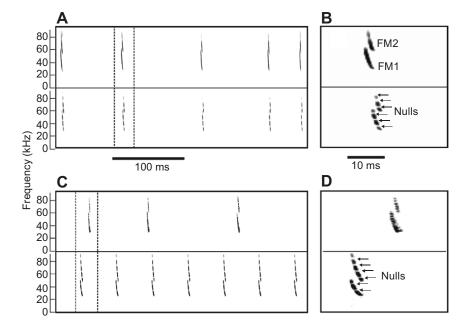d under a state scientific collecting permit. Because bats were wild-caught, their ages could not be ascertained. They were housed in individual cages in a temperature- and humidity-controlled colony room (22–24°C, 40–60% relative humidity) and kept on a reverse 12 h dark:12 h light circadian cycle. Bats were fed live *Tenebrio* larvae (mealworms) daily, at numbers that stabilized their body mass within the 15–19 g range, and had free access to vitamin-enriched water. They were not food deprived for experiments and received their daily food allotment during the psychophysical procedure. Experiments were conducted during the bats' subjective night, at the same time each day. Procedures complied with Principles of Animal Care, publication no. 86-23 (1985) of the US National Institutes of Health, and were approved by the Brown University Institutional Animal Care and Use Committee.

Psychophysical procedure
Experiments took place inside a net-enclosed space (2.75 m long, 2 m wide and 2.8 m high) within a larger acoustically shielded room (8 m long, 4 m wide, 2.8 m high), the walls and floor of which were lined with sound-absorbent foam (SONEX®, Pinta Acoustic, Minneapolis, MN, USA). Tests were conducted in open space because small enclosures preclude testing sensitivity to echoes. The background sound level within the experimental area was confined to ventilation noise (50–100 Hz, 60–70 dB SPL). Other ambient sound was below 30 dB SPL, the noise floor of the Bruel & Kjaer Type 4135 condenser microphone used for measurements. The experimental apparatus consisted of a Y-shaped platform (12 cm wide and 20 cm front to back) mounted at a height of 1.2 m on a heavy Brunson optical tooling stand. An electrostatic loudspeaker (RCA model 112343, Hauppauge, NY, USA), for presenting stimuli, and an ultrasonic microphone (FG-3329 electret microphones, Knowles Electronics, Itaska, IL, USA), for picking up the bat's sonar emissions, were mounted on the end of each Y-arm, level with the platform. The area in front and to the sides of the surveying stand was kept clear of other extraneous echo-producing obstacles. Equipment for presenting sounds and recording bat responses was located behind the stand, while electronic equipment for producing sounds was housed in an adjacent room. Light levels were dim (10–30 lx) to avoid disturbing the bats.

Fig. 1. Spectrograms of frequency modulated (FM) stimuli during active echolocation and passive hearing tasks. (A) A sequence of five FM broadcasts emitted by the bat during the active echolocation task (top), with the corresponding FM echoes (bottom). (B) Expanded view of spectrograms (outlined by vertical dashed lines in A), showing details of one active FM broadcast (top; two harmonics, FM1 and FM2) and its electronically delayed echo (bottom). Echo spectrograms show nulls (white horizontal slices through the spectrum at 10 kHz intervals; arrows) in both FM1 and FM2, indicating that the sound as a whole contains two-glint reflections 100 µs apart, simulating a target with glints separated by 1.7 cm in range. (C) Spectrograms of three FM broadcasts (top row) emitted by the bat during the passive hearing (detection) task, along with FM stimuli (bottom) passively delivered to the bat during a trial. While bats emit their own sounds sporadically during passive trials, they receive no active echoes, only the passive sweeps. (D) Expanded view (outlined by vertical dashed lines in C) showing one active FM broadcast (top) and one FM passive stimulus (bottom). The passive FM stimuli are similar in structure to the two-glint active FM echoes.

Bats were trained to sit at the center of the Y-shaped platform, to crawl forwards towards the right or the left arm in response to a stimulus from the electrostatic loudspeaker at that arm, and to eat a piece of mealworm from plastic forceps as a reward for each correct response. The method depended on each bat not flying away but remaining on the platform for the duration of daily sessions. Once the bats learned this initial task, psychophysical training began. Two different methods, an active echolocation task in which the bats echolocated to receive echoes and a passive hearing task in which synthetic FM sounds were presented without active echolocation, were used to estimate detection thresholds. Two bats (Doc and Weezer) trained in the active task learned to emit echolocation sounds in order to receive and detect electronically delayed echoes of their sonar emissions at different signal levels. Each emission was picked up by the ultrasonic microphone, sent to an electronic delay-line system, filtered, and delivered back to the bat through the relevant electrostatic loudspeaker at a delay of 3160 µs, corresponding to an echo from a virtual object located 54.5 cm from the end of the Y-arm on that side. Spectrograms of the bat's active emissions and the electronically delayed echoes are shown in Fig. 1A,B. Each echo contained two replicas of the broadcast separated by 100 µs to mimic the two-glint echoes typically received from 1–2 cm insect targets. This basic procedure has been used extensively to examine processing of biosonar sounds (Simmons, 1979; Bates et al., 2011).

Six bats (Weezer, Felix, Heisenberg, Mellie, Boo and Dandelion) were trained in the passive task to detect synthetic echoes, presented whether or not they echolocated. Trains of digitally generated downward-sweeping FM sweeps, mimicking echoes from a typical sonar broadcast, were presented in each trial. Each sweep (3 ms duration) consisted of two downward-sweeping harmonics (FM1: 55 to 22 kHz; FM2: 105 to 45 kHz). Each two-harmonic signal was delayed 100 µs and added to itself to match the two-glint structure of echoes in the active task (Fig. 1C,D). Stimuli were produced by passing the digitally generated FM sweeps through the same electronic delay-line system used to produce the active echoes, and were presented to the bat (at a repetition rate of 20 Hz) through the left or the right electrostatic loudspeaker. The ultrasonic microphones remained at the ends of the arms of the Y-shaped platform but were turned off so they could not feed extraneous signals into the delay lines that would corrupt the stimuli. Even though bats were not required to echolocate to obtain echoes, they occasionally did while on the platform (Fig. 1C,D).

A two-alternative forced-choice design and adaptive threshold-tracking procedure (Cornsweet, 1962) were used to determine detection thresholds. In each trial, the bat was required to detect a stimulus presented from either the right or the left loudspeaker. The side of stimulus presentation was pseudorandomized (Gellermann sequence; Gellermann, 1933), with the constraint that the stimulus could not be presented on the same side for more than three successive trials. The number of trials per day was limited by the number of food rewards an individual bat could be given in order to maintain its body mass in a healthy range, and so varied from bat to bat. Bats performed between 20 and 70 trials per day, depending on the animal's individual food allotment, how many correct and incorrect choices were made, and how many mealworm pieces a bat may have dropped. An individual trial lasted for less than 3 s (bats were given a maximum of 3 s to make a choice before the trial was aborted), so durations of daily testing sessions, including the time taken for the bat to eat its reward and to be moved back to the starting platform between trials, ranged from less than 3 min to as long as 10 min. In both active and passive tasks, stimulus levels were

initially set at 66 dB SPL rms and then varied downward according to the bat's performance. Each stimulus level was presented in a block of five trials. If the bat correctly detected the stimulus on at least four out of five (80% correct performance) of these trials, then the stimulus level was lowered by 3 dB and presented at that new level for a second block of five trials. If the bat made two errors (40% correct performance) in a five-trial block, then the stimulus level was increased by 1 dB for the next set of five trials. Blocks of three trials and steps of 2 dB were sometimes used, as identified in the figure captions. Beginning stimulus levels on any particular day depended on the bat's performance on the previous day. Experiments continued until bats reached a stable threshold for detection, defined as that stimulus level where performance was at the average reversal point between correct and incorrect responses (Gourevitch, 1970) over six testing days. Bats were tested for an average of 28 days before stable performance was reached.

Experiments were conducted in a double-blind manner. A 'trainer' handled the bat and administered food rewards for correct responses, and a 'recorder', sitting behind the trainer, controlled the order of stimulus presentations and monitored the bat's position on the platform from the display of a Sony digital 8 mm video Walkman® (New York, NY, USA) connected to a CCD video camera (type 166 15-CB22-1, Supercircuits, Inc., Austin, TX, USA)
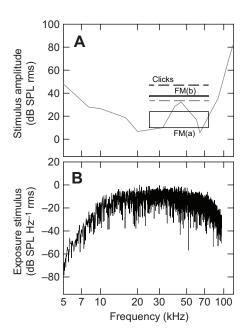

Fig. 2. The spectrum of the noise exposure stimulus parallels the hearing sensitivity of the big brown bat. (A) Audiogram of the big brown bat (solid line), averaged and replotted from Dalland (1965) and Koay et al. (1997). The audiogram shows mean thresholds to long duration pure tones ['continuous' in Dalland (1965) and 400 ms in duration in Koay et al. (1997); SPL, sound pressure level; rms, root mean square]. The audiogram is U-shaped, except for an area of elevated thresholds around 40 kHz produced by external ear effects. Thresholds to FM echoes are traced by the black-outlined rectangles. FM(a) encompasses the range of thresholds reported by Kick and Simmons (1984) and Simmons et al. (1992) in experiments where echo delay is varied. FM(b) encompasses the range of thresholds reported by Møhl and Surlykke (1989) to FM sweeps of 1.7–2 ms duration. Frequencies in FM sweeps extend from 25 to 75 kHz. Thresholds to clicks (replotted from Surlykke and Bojesen, 1996) are traced by the horizontal dashed lines. The range of psychophysical thresholds to FM sweeps obtained in the present experiment in both active echolocation and passive hearing falls within the range of thresholds to FM sweeps obtained in these earlier experiments. (B) Spectrum of broadband noise used as the exposure stimulus.

Table 1. Summary of testing conditions and threshold shifts for all bats

Psychophysical task	Bat	Pre-exposure threshold (dB SPL rms)	Post-exposure (dB shift)		
			20 min	2 h	24 h
Active echolocation	Doc	35 (1)	+4	–	–4*
		35 (2)	0	–	–1
	Weezer	23	+1	–	+1
	Weezer (sham)	26	0	–	–3
Passive detection	Felix	33 (1)	+5	–	–6
		29 (2)	–4	–	0
	Weezer	26 (1)	–2	–	–2
		22 (2)	0	–	+1
	Mellie	21	–	–2	+1
	Boo	27	–	–13	–5
	Heisenberg (sham)	31	–1	–	+3
	Mellie (sham)	22	–3	–	–2
	Dandelion (sham)	22	–	0	–2

Seven big brown bats were trained in an active echolocation (*N*=2) or a passive detection (*N*=6) task. Pre-exposure thresholds for detection of frequency modulated (FM) sweeps (echoes) are listed in the third column. Six bats were exposed to 116 dB sound pressure level (SPL) broadband noise for 1 h (152 dB sound exposure level, SEL), and four bats were sham-exposed for 1 h. Three bats (Doc, Felix and Weezer) were exposed to noise twice, as indicated by the numbers 1 and 2 (in parentheses) after their pre-exposure thresholds. Threshold shifts at post-exposure times of 20 min, 2 h and 24 h are listed for each bat. Positive numbers indicate higher thresholds after noise exposure and negative numbers indicate lower thresholds after noise exposure. Dashes indicate no data for that condition. *Insufficient trials for a clear threshold estimate (see Results).

mounted on the ceiling above the Y-shaped platform. The trainer had no knowledge of the order or intensity level of stimulus presentations. Trials were initiated by the recorder as soon as the trainer placed the bat on the starting end of the platform. To 'prime' the bat, the first three to five trials on each testing day were cued, with sounds on the right or the left presented simultaneously with a piece of mealworm. The remainder of the trials were not cued. When the bat made a correct response, the recorder informed the trainer by saying 'correct' and the trainer rewarded the bat with a piece of mealworm. The bat was allowed to eat its reward and was then removed from the platform. When the bat made an incorrect response, the recorder made a 'shh' sound to signal to the bat and the trainer that an error had occurred, and the bat was removed from the platform. Bats typically responded as soon as they were placed on the starting end of the platform. If a bat did not make a choice within 3 s, then the recorder indicated an incorrect response had been made, the bat was removed from the platform and a new trial commenced.

In control experiments, both loudspeakers were turned off without the prior knowledge of either the trainer or the recorder. Under these conditions, bats were expected to perform randomly.

Noise exposure
Once a bat reached a stable threshold, on the next testing day it was placed into a small (15×15×15 cm) steel mesh cage in a sound-attenuating chamber for either noise or sham exposures. Sham exposures control for transporting bats to a new location and allow assessment of threshold variability unrelated to noise exposure. Ambient sound in the chamber was less than 30 dB SPL at all frequencies from 50 Hz to 100 kHz. The spectrum of the broadband noise exposure stimulus ranged from 10 kHz to 100 kHz, spanning the audible range of the big brown bat (Dalland, 1965; Koay et al., 1997; Fig. 2). This stimulus was generated with an Elgenco analog random noise generator, filtered (Wavetek Rockland 442 filter, 10–100 kHz, roll-off 48 dB), attenuated, amplified (Harmon-Kardon PM645 power amplifier), and presented to the bat from a Panasonic EAS 10TH leaf tweeter. When bats were placed into the cage, they adopted a stable position either hanging upside-down from one of the walls or sitting on the floor, and they appeared to remain in their preferred positions for the duration of the exposure.
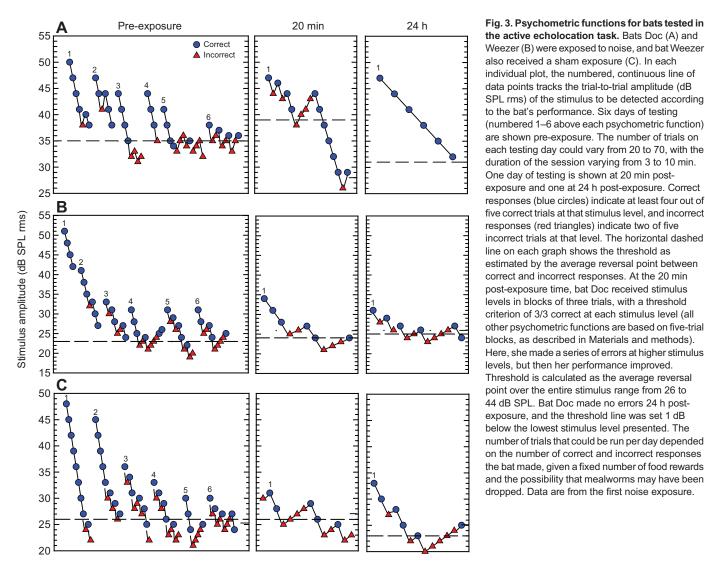
The loudspeaker was positioned so that it was oriented 20 cm away from and pointing directly towards the bat. Noise level was measured by a calibrated Brüel & Kjær model 4135 1/4 in condenser microphone placed along one wall of the mesh cage. It was set at a nominal value of 116 dB SPL (SEL of 152 dB re. 400 µPa^2 s; American National Standards Institute, 1994) at the amplifier and varied from 113 to 120 dB SPL at different positions in the cage. Noise or sham exposures lasted 1 h.

The amplitude and duration of noise exposures were chosen based on pilot experiments on two additional bats trained on the active echolocation task. In these experiments, noise exposures at levels of 80, 90 or 100 dB SPL for durations of 15, 30 or 45 min did not shift post-exposure thresholds (tested at 5 or 45 min) above pre-exposure thresholds for either bat. These pilot data were used to design the testing conditions in this report. Two bats trained on the active task were exposed to noise for 1 h and thresholds were re-measured beginning 20 min after the end of exposure and then 24 h post-exposure. Four bats trained on the passive task were exposed to noise and thresholds were re-measured beginning 20 min (*N*=2) or 2 h (*N*=2) post-exposure, and then 24 h (*N*=4) post-exposure. One bat trained on the active task and two bats trained on the passive task were not exposed to noise, and their thresholds were re-measured 20 min (*N*=2), 2 h (*N*=1) and 24 h (*N*=3) after these sham exposures. Post-exposure testing times of 20 min, 2 h and 24 h are within the range (4 min to 24 h) used in studies of TTS in other terrestrial mammals (Ryan and Bone, 1978; Heffner et al., 2008).

One bat was re-exposed 2 weeks after the first exposure and two bats were re-exposed to noise 6 weeks after the first exposure. Stimulus parameters for this second noise exposure were the same as those for the first.

Data presentation and analysis
Data are presented as psychometric functions showing correct and incorrect performance at each stimulus level on each testing day. Each individual plot (Figs 3–5) tracks the bat's performance (correct/incorrect responses) in pre-exposure testing (6 days) and post-exposure testing (1 day at each post-exposure time). Changes in hearing sensitivity are expressed as threshold shifts (dB) between pre-exposure and post-exposure thresholds (Table 1). Positive values

Fig. 3. Psychometric functions for bats tested in the active echolocation task. Bats Doc (A) and Weezer (B) were exposed to noise, and bat Weezer also received a sham exposure (C). In each individual plot, the numbered, continuous line of data points tracks the trial-to-trial amplitude (dB SPL rms) of the stimulus to be detected according to the bat's performance. Six days of testing (numbered 1–6 above each psychometric function) are shown pre-exposure. The number of trials on each testing day could vary from 20 to 70, with the duration of the session varying from 3 to 10 min. One day of testing is shown at 20 min post-exposure and one at 24 h post-exposure. Correct responses (blue circles) indicate at least four out of five correct trials at that stimulus level, and incorrect responses (red triangles) indicate two of five incorrect trials at that level. The horizontal dashed line on each graph shows the threshold as estimated by the average reversal point between correct and incorrect responses. At the 20 min post-exposure time, bat Doc received stimulus levels in blocks of three trials, with a threshold criterion of 3/3 correct at each stimulus level (all other psychometric functions are based on five-trial blocks, as described in Materials and methods). Here, she made a series of errors at higher stimulus levels, but then her performance improved. Threshold is calculated as the average reversal point over the entire stimulus range from 26 to 44 dB SPL. Bat Doc made no errors 24 h post-exposure, and the threshold line was set 1 dB below the lowest stimulus level presented. The number of trials that could be run per day depended on the number of correct and incorrect responses the bat made, given a fixed number of food rewards and the possibility that mealworms may have been dropped. Data are from the first noise exposure.

indicate that threshold increased after noise exposure and negative values indicate that threshold decreased after exposure. Statistical tests were performed using SPSS v. 22 (IBM SPSS, Armonk, NY, USA).

RESULTS
Performance in pre-exposure testing
Pre-exposure thresholds were estimated for each bat over a 6 day span of testing by averaging reversal points in the descending sequence of stimulus amplitudes as determined by the proportion of correct and incorrect responses. Over this time span, some bats (Fig. 3, Doc and Weezer; Fig. 4, Felix and Heisenberg) showed day-to-day variability of as much as 6 dB, while others (Fig. 4, Mellie; Fig. 5, Boo and Dandelion) were more stable. These data indicate that threshold shifts of 6 dB are within normal variability or measurement error for individual animals.

Estimated pre-exposure thresholds in active and passive tasks (Figs 3–5) ranged from 21 to 35 dB SPL (mean of 27 dB SPL) across the seven bats. Thresholds for the two bats tested in pilot experiments were 31 and 36 dB SPL. Repeated testing of four bats (Doc, Weezer, Felix and Mellie; Table 1) showed that threshold estimates for individuals are stable within 4 dB. Doc was tested in the active task and was exposed to noise twice, 2 weeks apart. Her pre-exposure threshold prior to both exposures was 35 dB SPL

(Fig. 3A, Table 1). Weezer was tested in active (Fig. 3B,C) and passive (Fig. 4B) tasks. His pre-exposure thresholds varied by 4 dB (23 dB SPL in active detection before noise exposure, 26 dB SPL in active detection prior to sham exposure, Fig. 3B,C; 26 dB SPL in passive detection prior to the first noise exposure, Fig. 4B; and 22 dB SPL in passive detection prior to the second noise exposure, Table 1). Pre-exposure thresholds for Felix were 33 dB SPL prior to the first exposure and 29 dB SPL prior to the second exposure (Table 1). Mellie's pre-exposure thresholds in two passive detection tests were 22 dB SPL (Fig. 4D) and 21 dB SPL (Fig. 5A, prior to noise exposure). These data confirm that threshold differences up to 4 dB are within the baseline performance variability for an individual bat.

On seven testing days (one active echolocation, six passive hearing), loudspeakers were turned off without the knowledge of either the trainer or the recorder. Bat performance suffered under these conditions (Doc, 54% correct; Felix, 43% correct; Dandelion, 63% correct; Weezer, 63% correct). These levels of performance are well below the 80% criterion used to define correct responses.

Performance after noise exposure
Thresholds for three bats tested in active detection were measured 20 min and 24 h after noise exposure (Fig. 3). Bat Doc's pre-
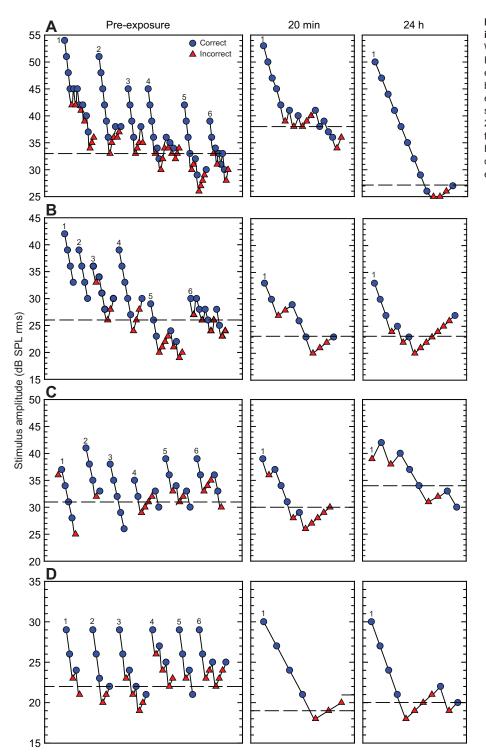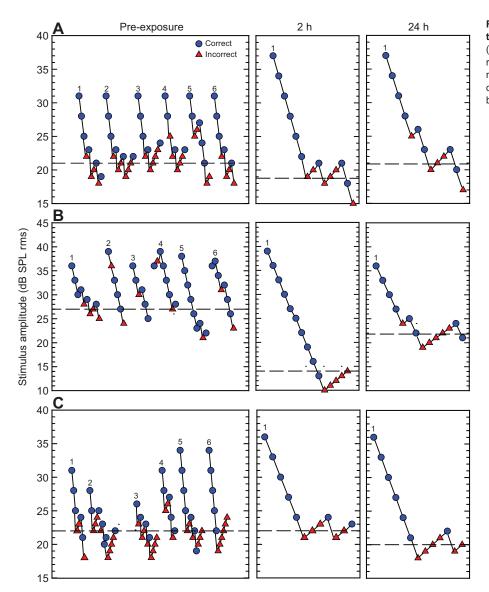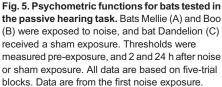
Fig. 4. Psychometric functions for bats tested in the passive hearing task. Bats Felix (A) and Weezer (B) were exposed to noise, while bats Heisenberg (C) and Mellie (D) received sham exposures. Thresholds were measured for 6 days before exposure, and then 20 min and 24 h after exposure or sham exposure. Bat Felix received stimuli in blocks of three trials at both 20 min and 24 h post-exposure. All other data are based on five-trial blocks. For bats Felix, Weezer and Heisenberg, stimulus levels were occasionally changed in 2 dB steps. Data are from the first noise exposure.

exposure threshold (Fig. 3A) was 35 dB SPL. When tested 20 min post-exposure, this bat made several errors initially in the testing session, but then her performance improved. Calculated using the mean of all the reversal points throughout the entire testing session, Doc's threshold was 39 dB SPL, indicating a 4 dB loss of hearing sensitivity 20 min after noise exposure. If only her performance in the first half of testing is considered, then this bat may have suffered a 7 dB loss of sensitivity (calculated threshold of 43 dB SPL compared with the pre-exposure threshold of 35 dB SPL) that then very quickly recovered to a threshold of 28 dB SPL, as calculated

from the reversal points at the end of the testing session. The use of all reversal points over the entire testing session, and not just the initial ones, provides a fuller estimate of this bat's hearing sensitivity after noise exposure. Doc's threshold 24 h post-exposure could not be calculated because it was not possible to conduct enough trials as a result of limitations in food allotment; it was estimated to be 31 dB SPL, 1 dB lower than the level of the last correct response, and within 3 dB of the final threshold obtained in the second half of the 20 min post-exposure testing session. Bat Weezer's performance was more stable (Fig. 3B). His post-exposure thresholds differed

Fig. 5. Psychometric functions for bats tested in the passive hearing task. Bats Mellie (A) and Boo (B) were exposed to noise, and bat Dandelion (C) received a sham exposure. Thresholds were measured pre-exposure, and 2 and 24 h after noise or sham exposure. All data are based on five-trial blocks. Data are from the first noise exposure.

from his pre-exposure threshold by 1 dB (20 min) and 2 dB (24 h). After sham exposure (Fig. 3C), Weezer's threshold was stable at 20 min, and decreased by 3 dB at 24 h. The mean threshold shift 20 min after noise exposure for these two bats was 2.5 dB, and the mean shift 24 h post-exposure was −1.5 dB.

Two bats (Felix and Weezer) were trained in the passive task and then exposed to noise (Fig. 4A,B). Felix showed a 5 dB increase in threshold 20 min after exposure, and a 6 dB decrease in threshold 24 h after exposure. Weezer's thresholds decreased by 2 dB after noise exposure. The mean threshold shift at 20 min post-exposure for these two bats was 1.5 dB, and the mean threshold shift at 24 h post-exposure was −4 dB. The mean threshold shift at 20 min post-exposure for the four bats tested in active and passive tasks was 2 dB, not significantly different from the null hypothesis of 0 dB shift (one-sample t-test, $P>0.05$).

Detection thresholds for bats Mellie and Boo were tested 2 and 24 h after noise exposure (Fig. 5A,B). Mellie's threshold 2 h post-exposure was 19 dB SPL, 2 dB lower than her pre-exposure threshold. At 24 h post-exposure, her threshold returned to the pre-exposure level of 21 dB SPL. Boo was 13 dB more sensitive 2 h after noise exposure than she was before exposure; 24 h later, however, her threshold increased by 8 dB, to a level 5 dB lower than

her pre-exposure threshold. For these two bats, the mean threshold shift at 2 h post-exposure was −7.5 dB, indicating an increase in hearing sensitivity, but the difference between the bats was large. The mean threshold shift at 24 h post-exposure was −2.5 dB. The threshold shift 24 h post-exposure for the six bats tested in both detection tasks was −2.6 dB, not significantly different from the hypothesized 0 dB shift (one-sample t-test, $P>0.05$).

Performance after sham noise exposure

Thresholds from four bats subjected to sham exposures varied up to ±3 dB from pre-exposure thresholds (Table 1). The mean threshold shift at 20 min post-sham exposure was −1.3 dB (bats Weezer, Heisenberg and Mellie, in active and passive tasks), while at 24 h post-sham exposure, the mean threshold shift was −1 dB (bats Weezer, Heisenberg, Mellie and Dandelion, in active and passive tasks). These threshold changes are not significantly different from 0 dB (one-sample t-test, $P>0.05$).

Performance after a second noise exposure

Three bats (Doc, Felix and Weezer) were re-exposed to noise 2 or 6 weeks after the first exposure (Table 1). Doc exhibited the same pre-exposure threshold of 35 dB SPL after initial training and over

the 6 days prior to the second exposure, 2 weeks later. Her threshold 20 min after the second exposure did not change from her pre-exposure threshold, and her threshold 24 h later differed by only 1 dB. These changes in threshold are smaller than the 4 dB threshold shift seen after the first noise exposure. Felix and Weezer were re-exposed to noise 6 weeks after the initial exposure. Calculated thresholds prior to this second exposure were 4 dB lower for both bats compared with their thresholds prior to the first exposure. Neither bat showed threshold increases 20 min or 24 h after the second exposure.

Averaging data over the first and second noise exposures for all bats, the mean threshold shift 20 min post-exposure was 0.6 dB, and the mean shift 24 h post-exposure was −1.7 dB. These threshold changes are not significantly different from 0 dB (one-sample t-test, $P>0.05$).

DISCUSSION
No significant TTS in big brown bats
The results of these experiments demonstrate that big brown bats do not suffer significant threshold increases, outside the 3–6 dB range of baseline variability, in response to either FM echoes (active echolocation) or synthetic FM sweeps (passive hearing) after exposure to broadband noise at the level (152 dB SEL) and frequency band used in this study. The FM sweeps the bats were trained to detect are similar in acoustic structure to echoes received from insect targets during nightly foraging. Noise parameters are within the acoustic range (level, bandwidth) that bats could encounter in their natural environments. Post-exposure thresholds were assessed at time intervals within the range of those used to assess TTS in terrestrial mammals. The results are consistent with the hypothesis that big brown bats are less susceptible to noise-induced hearing losses than expected on the basis of broadband noise exposure data from other mammals. The bats' decreased susceptibility may be related to the unique demands of echolocation, which requires foraging and navigation in the midst of intense noisy soundscapes.

Reliability of measurements of hearing sensitivity
Comparisons of our estimates of hearing sensitivity with those obtained in independent experiments with this species confirm the reliability of our threshold measurements. In active echolocation, detection thresholds have been shown to depend on the duration of the FM broadcasts, the delay of echoes and the task the bat is asked to perform (Kick, 1982; Kick and Simmons, 1984; Simmons et al., 1992). Thresholds measured in these previous experiments varied from 0 to 35 dB SPL rms depending on these experimental parameters, and by as much as 10–13 dB from bat to bat. Using another detection task, Møhl and Surlykke (1989) estimated thresholds to 2 ms duration FM sweeps to be 37 and 38 dB SPL. Surlykke and Bojesen (1996) reported thresholds of 47 dB SPL to 1 ms duration clicks, and thresholds of 33.5 dB SPL to trains of 20 clicks, which are higher than the mean threshold calculated from our data. Finally, ABR thresholds to FM sweeps hovered around 40 dB SPL in two different experiments (Simmons et al., 1990; Burkard and Moss, 1994). These comparisons indicate that our procedures provided appropriate estimates of bat hearing sensitivity for echoes.

Pure tone audiograms for the big brown bat show thresholds around 7–12 dB SPL in the most sensitive frequency range of 20–30 kHz (to continuous tones, Dalland, 1965; to 400 ms tones, Koay et al., 1997), but these audiograms are based on thresholds to tones considerably longer in duration than the 3 ms FM sweeps used in our experiments. The relatively long duration of these tones ensures

concentration of energy at a single frequency, while in 3 ms FM sweeps, the effective duration of any one frequency is limited to about 100 µs. It is well known from mammalian psychoacoustics that detection thresholds vary with stimulus duration (Meddis and Lecluyse, 2011), particularly the sound's dwell-time at specific frequencies. On duration versus energy considerations alone, each of the tonal stimuli in Koay et al. (1997) deliver up to 400 times more energy than each entire FM sound used in our experiments, for an expected threshold elevation of up to 26 dB for the FM sounds.

Pre-exposure thresholds of individual bats to FM sweeps varied by 14 dB, about the same range as obtained in previous studies using a similar two-choice procedure (Simmons et al., 1992). This variability could reflect age differences between wild-caught animals, differences in their individual criteria for making choices, limitations in numbers of food rewards that could be offered on any given day, or experimental error. Two earlier psychophysical studies of echo detection in the same bat species found variability between individual bats of 10–13 dB (Kick and Simmons, 1984; Simmons et al., 1992). Individual variability in psychophysical thresholds to single frequencies has been commonly observed in studies of other mammals (Heffner et al., 2008; Finneran and Schlundt, 2013). In male college students, for example, Ward et al. (1958) found threshold differences between individuals of 10–37 dB at particular tone frequencies.

Performance of bats in initial threshold determination could vary from day to day, with some animals showing differences as large as 6 dB and others showing differences of around 3 dB (the step size used to sample stimulus levels). Several bats were tested several weeks apart, and for these animals, thresholds in pre-exposure varied by as much as 4 dB between the first and the second determination. Combined with the 6 dB variability sometimes seen over the course of pre-exposure threshold determination, these data suggest that variability of 3–6 dB is within the normal range of performance of bats in these experiments. In marine mammals, Finneran and Schlundt (2013) and Popov et al. (2013) both adopted a criterion of ±5 dB to monitor threshold stability, while in laboratory-bred rats (*Rattus norvegicus*), Heffner et al. (2008) used a criterion of 3–5 dB to indicate threshold stability and good correspondence between behavioral and electrophysiological measures of hearing sensitivity. The variability in our threshold measurements is consistent with these criteria.

Criteria for TTS
In marine mammals, TTS has been defined as threshold increases exceeding 6 dB (Finneran and Schlundt, 2013; Finneran, 2015), which is just above the baseline variability in responding in these experiments. By this criterion, which is also just above baseline variation in our study, big brown bats did not suffer TTS to the broadband noise exposure stimulus. The mean threshold shift (averaged over the first and second exposures) at 20 min post-exposure was 0.6 dB (range −4 to +5 dB). Within this range, one bat showed a threshold increase of 5 dB and another showed a threshold increase of 4 dB. Although these shifts are larger than those shown by the other bats, they do not reach or exceed the 6 dB criterion. The mean threshold shift 24 h post-exposure (all bats) was 0.8 dB, with the highest increase being 1 dB. These threshold shifts are not significantly different from those observed after sham noise exposures (−0.3 dB and −0.75 dB), and they are within the range of baseline variability in responding. It is possible that hearing losses of less than 6 dB could have occurred, but losses of this minimal amount would be difficult to discern.

There is no clear relationship between pre-exposure thresholds and amount of threshold shift after exposure. Two bats with the highest pre-exposure thresholds of 33 and 35 dB SPL showed threshold shifts of 4 and 5 dB at 20 min after the first noise exposure, but shifts of 0 and −4 dB after the second exposure. Bats with the lowest pre-exposure thresholds of 22 and 23 dB SPL showed threshold shifts of 0 and 1 dB after one exposure. Testing of more animals with greater variation in pre-exposure thresholds will allow clearer determination of any relationship between baseline hearing sensitivity and changes in sensitivity after noise exposure.

Individual variability in the amount and direction of the threshold shift was apparent, as has also been observed in other studies (Heffner et al., 2008). One bat exposed to noise twice showed a threshold increase of 5 dB, 20 min after the first exposure, but a threshold decrease of 4 dB after the second exposure. One other bat, available for these experiments only for a limited time, showed a stable threshold in pre-exposure testing but an unusual, transient threshold decrease of 13 dB at 2 h post-exposure that was not seen 24 h later. The amount of variation for the first bat did not exceed baseline levels, and that for the second bat was observed at one time point only. It is unclear whether these data reflect measurement error, a change in the animal's criterion for responding, or some other factor.

Three bats whose hearing was assessed at 20 min post-exposure all showed some errors at or near the beginning of testing on that day, which was then followed by a series of correct responses at lower stimulus levels until errors again appeared. It is possible that these initial errors represent some hearing loss that then quickly recovered over the course of threshold determination. This possibility is most evident in the data from bat Doc, whose performance early in testing suggests an initial 7 dB loss of sensitivity but over the entire testing session suggests a smaller loss of 4 dB. Because of the short duration of daily testing, this apparent recovery is indeed very rapid. Note, however, that even some control bats, not exposed to noise, showed some, albeit fewer, initial errors during threshold determination at the 20 min post-sham exposure time. One explanation for this phenomenon stems from our observation that the behavior of some bats, when returned to the platform after being placed in the sound-attenuating chamber, indicated reluctance to perform. This suggests that noise exposure might have affected their motivation. Videotape monitoring of some bats during the exposure time suggested that they were sleeping. Threshold testing at shorter post-exposure times and monitoring of all bats during exposures are needed to evaluate the source of the initial errors in performance.

TTS in other mammals

In other mammals, stimulus parameters including exposure bandwidth, level, duration and post-exposure testing time affect TTS induction and recovery (Ward et al., 1958; Clark, 1991; Finneran, 2015). Notwithstanding some differences in these parameters, comparisons of the performance of big brown bats with that of terrestrial mammals exposed to similar bandwidths and similar or even lower noise SELs indicate that bats suffer less TTS than expected. Ward et al. (1958) measured detection thresholds in humans after exposure to broadband noise (0.75–10 kHz; 88–100 dB SPL). After 102 min duration exposure to 100 dB SPL noise (138 dB SEL), the mean threshold shift to a 4 kHz tone, within the most sensitive region of the human audiogram, at 2 min post-exposure was 29.6 dB. Ward et al. (1958; see also Mills et al., 1981) proposed a model for predicting TTS growth and recovery in humans after noise exposures of various bandwidths, durations and

levels. Using this model to predict TTS in big brown bats to FM sweeps gives an expected threshold increase in excess of 40 dB. Because of the different stimulus parameters used in our experiments, these comparisons are only suggestive. But they do support the hypothesis that big brown bats may be comparatively less susceptible to TTS.

Data from other species also show differences between bats and other mammals in the magnitude of threshold increases after exposure to broadband noise. Nielsen (1982) measured psychophysical thresholds to 4 kHz tones in squirrel monkeys (*Saimiri sciureus*) 4.5 min after exposure to broadband noise (bandwidth 0.1–8 kHz, 95 dB SPL, duration 1 h; 131 dB SEL). The mean threshold increase was 10 dB. Mongolian gerbils exposed to two-octave noise (1414–5656 Hz, 110 dB SPL, 1 h; 146 dB SEL) showed 40–60 dB increases in thresholds to single tones at 30 min post-exposure (Ryan and Bone, 1978). Rats exposed to high-intensity tones (11–16 kHz, 120 dB SPL, 10 min; 156 dB SEL) experienced behavioral threshold increases between 10 and 60 dB, assessed 1 h post-exposure (Heffner et al., 2008). Shone et al. (1991) reported ABR threshold increases in mice (CBA/Ca) of 30–35 dB (1 h post-exposure) in response to high-frequency tones after exposure to broadband noise (0.5–40 kHz, 101 dB SPL, 45 min; 135 dB SEL). These threshold increases are all higher than the maximum 5 dB increase shown by big brown bats in response to noise at a SEL of 152 dB.

Like echolocating bats, cetaceans have evolved to live in environments where they are exposed to their own intense sonar emissions as well to those of conspecifics (Nachtigall and Schuller, 2014). At SELs greater than 160 dB, different cetacean species exhibit TTS ranging from 7 to 63 dB depending on the specific testing conditions (Finneran, 2015). TTS in dolphins (*Tursiops truncatus*) and beluga whales is greater for lower (10–30 kHz) than for higher (90 kHz) frequencies (Finneran and Schlundt, 2013; Popov et al., 2013), suggesting some relationship between the spectral content of echolocation sounds and susceptibility to noise-induced hearing losses. These studies have focused on quantifying hearing losses to intense tones or narrow band noise, especially within the frequency range of anthropogenic noise sources, rather than to broadband noise and/or to the animals' own sonar emissions. But these comparisons raise the hypothesis that echolocating mammals are less susceptible to TTS than are non-echolocating mammals.

Limitations

Our data do not imply that bats will not suffer TTS under some experimental conditions, only that the stimulus parameters used in this study were not sufficient to produce it. Because we are interested in how bats cope with the intense, broadband sounds they experience in their natural environment, we did not measure thresholds to pure tones or to narrow band noise. These experiments will be important for determining whether bats, like mammals (Clark, 1991), suffer hearing loss at specific tone frequencies following narrowband noise exposures. The range of stimulus parameters that could be manipulated to assess TTS is enormous, and further research is required to confirm and to model the limits of bats' susceptibility to TTS. In particular, measuring hearing sensitivity at very short time intervals (2–4 min) after noise exposure will be crucial for understanding differences in susceptibility to noise-induced hearing losses between bats and other vertebrates. And although the sample sizes used here are at the high end of the numbers typically used in studies of echolocating animals, testing of more animals is warranted.

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