

## RESEARCH ARTICLE

# Echolocation behavior in big brown bats is not impaired after intense broadband noise exposures

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## ABSTRACT

Echolocating bats emit trains of intense ultrasonic biosonar pulses and listen to weaker echoes returning from objects in their environment. Identification and categorization of echoes are crucial for orientation and prey capture. Bats are social animals and often fly in groups in which they are exposed to their own emissions and to those from other bats, as well as to echoes from multiple surrounding objects. Sound pressure levels in these noisy conditions can exceed 110 dB, with no obvious deleterious effects on echolocation performance. Psychophysical experiments show that big brown bats (*Eptesicus fuscus*) do not experience temporary threshold shifts after exposure to intense broadband ultrasonic noise, but it is not known if they make fine-scale adjustments in their pulse emissions to compensate for any effects of the noise. We investigated whether big brown bats adapt the number, temporal patterning or relative amplitude of their emitted pulses while flying through an acoustically cluttered corridor after exposure to intense broadband noise (frequency range 10–100 kHz; sound exposure level 152 dB). Under these conditions, four bats made no significant changes in navigation errors or in pulse number, timing and amplitude 20 min, 24 h or 48 h after noise exposure. These data suggest that big brown bats remain able to perform difficult echolocation tasks after exposure to ecologically realistic levels of broadband noise.

**KEY WORDS:** Biosonar, Flight, Hearing impairment, Sonar sound groups, Temporary threshold shift

## INTRODUCTION

Big brown bats [*Eptesicus fuscus* (Palisot de Beauvois 1769)] emit trains of intense frequency-modulated (FM) biosonar pulses and use the information from returning echoes to analyze the surrounding acoustic scene. These bats dynamically change the duration, spectrum, directional aim, number and temporal patterning of their emitted pulses, and thus the acoustic sampling of the spatial scene, to reflect the complexity of their surroundings (Surlykke and Moss, 2000; Moss et al., 2006; Moss and Surlykke, 2010; Petrites et al., 2009). In particular, the time intervals [inter-pulse intervals (IPIs)] between the individual pulses in a train are known to vary with the perceived difficulty of the experimental task and according to the bat's individual strategy for navigating the scene (Surlykke and Moss, 2000; Petrites et al., 2009; Barchi et al., 2013; Falk et al., 2014; Kothari et al., 2014; Sändig et al., 2014; Knowles et al., 2015;

Wheeler et al., 2016). Changes in IPIs thus provide one index of the bat's vocal adaptations to its surroundings, particularly when steering through and foraging within cluttered acoustic environments.

One contributor to the complexity of the bat's acoustic environment is the presence of other bats. Many species, including big brown bats, are social animals that live in colonies and fly in groups (Davis et al., 1968; Simmons et al., 2004). Biosonar emissions from groups of bats exiting a roost or foraging together can reach aggregate sound pressure levels (SPL; re. 20  $\mu$ Pa rms) of 110–140 dB (Jakobsen et al., 2013; Kloepper et al., 2016). In spite of the potential for masking from the signals of conspecifics as well as obstacle echoes, in their natural environments bats orient, navigate and catch small insect prey successfully. The success of echolocation within such acoustically cluttered conditions indicates that rejection of deleterious impacts from intense noise exposures typically occurs as part of the bat's natural behavioral repertoire.

In many vertebrates, prior exposure to intense noise induces temporary threshold shifts (TTS), transient losses of hearing sensitivity that extend beyond the duration of the exposure itself. The amount of TTS and the time course of hearing recovery depend on the frequency bandwidth, level and duration of the preceding exposure (Finneran, 2015). Psychophysical experiments (Simmons et al., 2016) show that big brown bats do not suffer TTS, defined as threshold increases exceeding 6 dB, after extended exposure to intense ultrasonic broadband noise at a level and frequency range that could be encountered in the natural environment. In contrast, rodents, primates and humans all suffer TTS after comparable levels and durations of exposure to broadband noise within these species' hearing ranges (Ward et al., 1958; Mills et al., 1981; Nielsen, 1982; Shone et al., 1991). Previously, we suggested that terrestrial species may be more impacted by noise exposures because, unlike echolocating bats, they largely have evolved in quiet environments where natural occurrences of prolonged, intense sounds are rare (Simmons et al., 2016).

It is possible that noise-induced disruption of bats' auditory capabilities could exist, and be manifested not as a loss of hearing sensitivity *per se* but instead as a loss of perceptual acuity for acoustic features in returning echoes. Such a loss in acuity might decrease the successful execution of behavioral tasks that require echolocation, such as navigating in acoustically cluttered environments. In this experiment, we challenged big brown bats with a difficult flight task – flying in dense, extended clutter – both before and after exposure to intense noise. We hypothesized, consistent with psychophysical experiments showing no TTS after identical noise exposures (Simmons et al., 2016), that perceptual acuity and flight performance would not be impaired by these prior noise exposures.

## MATERIALS AND METHODS

### Animals

Four adult big brown bats (three male, one female) were captured in the wild from buildings in Rhode Island under a scientific collecting

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permit issued by the state Department of Environmental Management. They were maintained in individual cages in a temperature- and humidity-controlled colony room (22–24°C and 40–60% relative humidity). The colony room was kept on a reverse light cycle (12 h dark:12 h light). Experiments were conducted during the bats' subjective night. Bats had free access to vitamin-enriched water, and were fed live mealworms (larval *Tenebrio molitor*) with daily rations adjusted to keep individual body mass in a healthy range between 15 and 19 g. All husbandry and experimental procedures complied with Guide for the Care and Use of Laboratory Animals: eighth edition, National Research Council of the National Academies of Sciences USA (2011), and were approved by the Brown University Institutional Animal Care and Use Committee.

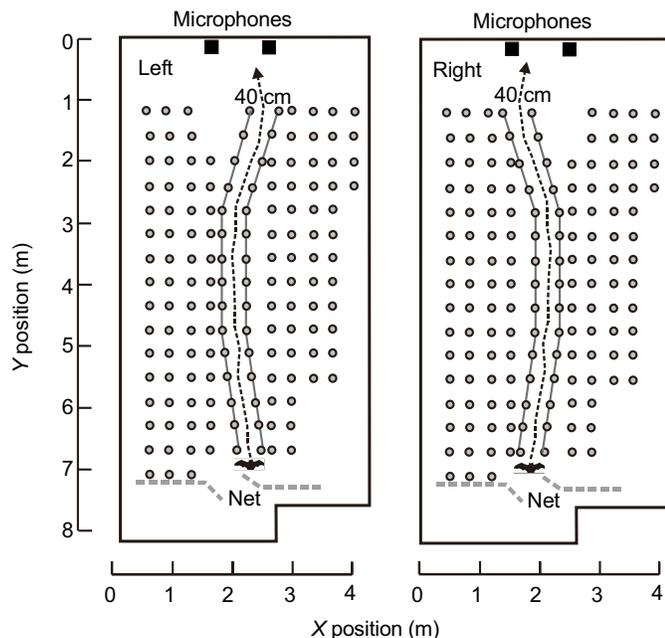
### Experimental paradigm

Experiments took place in a custom-built flight room (8.3 m long by 4.3 m wide by 2.7 m high) insulated acoustically and electrically from external noise. Walls and ceiling of the room were entirely covered in fireproof, anechoic acoustic foam (SONEX®, Pinta Acoustic, Minneapolis, MN, USA) that dampened any residual wall reflections by 20–25 dB, and the floor was carpeted. The flight room was filled with rows of closely spaced black plastic chains extending vertically from ceiling to floor (plan view, gray circles in Fig. 1), arranged to leave a narrow (40 cm wide), curved corridor through which the bats could fly. This basic chain configuration, but arranged for straight or curved corridors of different widths and

chain densities, has been used previously to examine echolocation during flight in the absence of prior noise exposures (Petrites et al., 2009; Knowles et al., 2015; Wheeler et al., 2016). Individual chain links measured 4.0 cm wide, 7.5 cm long and 1.0 cm thick, and are strong reflectors of the bat's incident emissions (Petrites et al., 2009) and resemble vegetation-like acoustic reflectors encountered in the natural environment. Chains were spaced 30 cm apart in rows and successive rows were separated by 40 cm, the same as the high-density chain configuration used in Petrites et al. (2009). This dense configuration creates a complex acoustic echo scene combining proximity, density and spatial extent, and it challenges the bat's biosonar capabilities for flight guidance (Barchi et al., 2013). The 40 cm corridor width was used for this experiment because it is difficult for the bat (Wheeler et al., 2016): the maximum wingspan of the big brown bat is 30–32 cm (Sändig et al., 2014), leaving about 4–5 cm of space on either side of the animal when flying through the corridor. Flight through such a narrow corridor would be expected to show a strong impact of noise exposure, if any occurred.

All four bats had substantial prior experience flying through straight corridors (widths 40 to 100 cm) in this chain array (Wheeler et al., 2016), so no progressive familiarization with the flight task itself over test days interfered with any effects of the noise exposure. Bats were flown through the array on four consecutive test days (pre-exposure, and 20 min, 24 h and 48 h post-exposure). The shape of the corridor alternated between left and right curvatures from one test day to the next (Fig. 1), preventing the formation of a stereotyped spatial map of the array (Barchi et al., 2013). The flight room was kept dark except for a small dim (90 lx) light near the corridor entrance. On each test day, the bat was released by experimenter 1 by hand from a fixed point at the entrance. The first test day was a pre-exposure (baseline) day, during which each bat was randomly assigned to fly through either a left or a right curved corridor. On the second test day, the bat was exposed to noise, as described below, and then 20 min later was flown through the array with the opposite corridor curvature. Each bat was flown again through the array on two subsequent days, 24 h and 48 h after noise exposure, with the corridor curvature alternating on each day. The post-exposure time points of 20 min, 24 h and 48 h are the same as those used in our previous psychophysical experiments (Simmons et al., 2016). On each test day, the bat was flown through the array for a total of 15–22 flights (Table 1). If the bat navigated the corridor without striking or colliding with any of the chains and then landed on the back wall of the flight room, it was rewarded by experimenter 2 with a piece of mealworm. These flights are called successful flights. Unsuccessful flights, in which the bat collided with a chain or fell to the floor before reaching the end of the corridor, were not rewarded. Any flights, successful or unsuccessful, marred by experimenter error or equipment malfunction were eliminated.

On the day of noise exposure, each bat was placed in a steel mesh cage (15 cm<sup>3</sup>) in a sound-attenuating chamber, elevated 1.5 m from the chamber's floor. A loudspeaker (EAS 10TH leaf tweeter, Panasonic, Osaka, Japan) was positioned 8 cm from the center of the mesh cage to one of its sides, and was oriented to point directly towards the bat. During the noise exposure, each bat typically adopted a stable position, either hanging on one side of the cage or resting on the floor. Noise was generated (Elgenco analog random noise generator), band-pass filtered (10–100 kHz, roll-off 24 dB/octave; Rockland 422 dual Hi/Low filter, Wavetek, San Diego, CA, USA), amplified (P645 power amplifier, Harmon-Kardon, Stamford, CT, USA), and sent to the loudspeaker. The level of noise in the center of the mesh cage was 116 dB SPL, as measured with a 1/4-inch condenser microphone (model 4135, Brüel and Kjær,



**Fig. 1. Two plan views of the flight room.** The left view shows the left-curving corridor and the right view shows the right-curving corridor. The corridor was fixed at a 40 cm width, and curved to the left or the right alternately on successive days for each individual bat. In each plan view, the release point is shown by the bat image, the positions of the chains are shown by the gray circles, the locations of the two microphones are shown by the black squares, and the bat's flight path is shown by the black dashed lines. The thick gray dashed lines near the release point demarcate the position of a hanging net that prevented the bat from flying outside the boundaries of the chain array. The two rows of chains making up the left and right boundaries of the corridor were suspended from flexible plastic pipes (light gray lines) so that the direction of the curve could be changed simply by moving the pipes, not by rehangings the individual chains.

**Table 1. Unsuccessful, successful, eliminated and total flights for each bat on each of the four test days relative to noise exposure**

Bat	Test day	Unsuccessful flights	Successful flights	Eliminated flights	Total flights
Gwen	Pre-exposure	4	15	1	20
	20 min post	3	16	3	22
	24 h post	2	15	2	19
	48 h post	0	15	2	17
Rev3	Pre-exposure	1	15	0	16
	20 min post	1	15	1	17
	24 h post	6	14	0	20
	48 h post	2	14	2	18
Icarus	Pre-exposure	0	17	0	17
	20 min post	6	13	1	20
	24 h post	2	16	1	19
	48 h post	1	12	3	16
Rameses	Pre-exposure	0	15	0	15
	20 min post	0	14	1	15
	24 h post	1	15	0	16
	48 h post	0	15	0	15

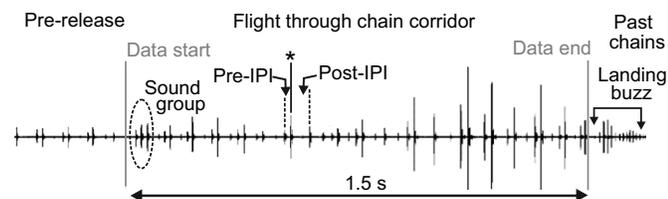
On unsuccessful trials, the bat collided with one of the chains or fell to the floor; these flights were not rewarded. On successful flights, the bat navigated the corridor without collisions; these flights were rewarded. Eliminated flights are those in which experimenter error or equipment malfunction occurred. Exposure to broadband noise did not increase significantly the numbers of unsuccessful flights.

Naerum, Denmark), but could vary from 3 dB higher to 2 dB lower at different locations inside the cage. The exposure lasted for 1 h, giving a total sound exposure level (SEL) of 152 dB SEL (re. 400  $\mu\text{Pa}^2$  s; American National Standards Institute, 1994). These exposure parameters are identical to those used to measure TTSs in a psychophysical procedure (Simmons et al., 2016).

### Sound recording and analysis

During each flight, the trains of pulses emitted by each bat were recorded with two ultrasonic microphones (SMG-0291, Knowles Electronics, Itasca, IL, USA; Fig. 1) mounted on custom-built preamplifier boards suspended at the end of the corridor. The microphones were positioned so that they could record all of the bat's pulses from the time of release until the time of landing on the back wall. The recorded pulses (Fig. 2) were digitized at 192 kHz (Model 702T, Sound Devices, Reedsburg, WI, USA) and saved as stereo .wav files. Microphone recordings were manually started by experimenter 2 before the release of the bat by experimenter 1, and then manually stopped by experimenter 2 after the bat had landed on the wall. Off-line analyses of the recorded sounds were performed using custom-written MATLAB procedures (R2014a, MathWorks,

Cambridge, MA, USA). Only recordings from microphone 1 were analyzed because those from microphone 2 were identical. For each flight, the recordings were first digitally high-pass filtered at 15 kHz to remove ambient noise. 'Flight audio trials' representing the duration of the flight down the corridor were selected from the entire audio recording of each trial (the entire recording included pulses emitted by the bat before it was released, the landing buzz and any pulses emitted by the bat after it had landed, and was typically around 2 s in duration). The end point of the flight audio trial was defined by the onset of the landing buzz, identified as a sudden and persistent drop in pulse amplitude and an increase in repetition rate occurring at the time the bat landed on the wall (Fig. 2). Moving backward in time from the first pulse in the landing buzz, a time interval of 1.5 s was selected for analysis. This 1.5 s interval covers the time needed for a bat to fly down to the end of the corridor after being released (Wheeler et al., 2016), but it does not include any pulses emitted by the bat prior to being released. The landing buzz itself was not included because the bat was already out of the chain array when the buzz occurred. In each flight audio trial, the time of each individual pulse was marked as the point at which its envelope reached its maximum amplitude. IPIs were calculated as the time intervals between the amplitude maxima of preceding and following pulses. For each individual pulse, IPIs were labeled as 'pre-IPI' (the IPI before the pulse) and 'post-IPI' (the IPI after that pulse; Fig. 2), and were measured for all but the first (no pre-IPI) and last (no post-IPI) pulses. Patterning of IPIs was analyzed using two metrics. First, we identified 'sonar sound groups' or 'strobe groups' (Moss et al., 2006; Petrites et al., 2009; Kothari et al., 2014), which are groups of pulses with short, stable IPIs surrounded by longer IPIs. Using the criteria formulated by Kothari et al. (2014), sound groups were classified as singles, doublets, triplets or quadruplets based on an 'island' criterion (between-group IPIs at least 20% larger than within-group IPIs) and a 'stability' criterion (5% tolerance within a group, for groups of three or more pulses). Because the cut-offs for these island and stability criteria are arbitrary and may not capture sound group patterning in all echolocation tasks, we also used a second metric based on the ratio of the post-IPI to the pre-IPI for each pulse (Wheeler et al., 2016). This ratio metric treats successive inter-pulse intervals in proportion so that, if the bat were to change the absolute size of the IPIs, this metric would still capture their proportional relation. It allows analysis of how intervals before and



**Fig. 2. Time-series waveform showing the series of pulses emitted by one bat while flying down the corridor on the pre-exposure day.** A time interval of 1.5 s (solid black horizontal line) prior to the landing buzz and after the bat was released by the experimenter was selected to define a 'flight audio trial'. The start and end of this time interval is indicated by the gray vertical lines. The landing buzz, a rapid burst of low-amplitude pulses after the end of the flight audio trial, indicates that the bat has landed on the wall. The time of each individual pulse (example designated by the vertical solid black line and asterisk) is characterized by a pre-inter-pulse interval (pre-IPI) and a post-IPI interval (dotted lines and arrows). The timing of the individual pulses shows that IPIs often alternate from short to long and *vice versa*. The dashed oval at the start of the flight audio trial shows a sonar sound group, in this case a triplet.

after each pulse are related to one another and does not rely on a particular definition of a sonar sound group.

Absolute amplitudes of pulses could not be measured because only two microphones positioned at the end of the corridor were used. Because of the decreasing distance between the bats and the microphones during flights, pulse amplitude seems to increase as the bat nears the end of the corridor. Relative amplitudes of pulses could be measured, and were calculated by normalizing successive pulse amplitudes to the amplitude of the first pulse in the flight audio trial on the pre-exposure day for that individual bat. These relative amplitudes can be compared between test days for an individual bat but not between bats. No compensation for spreading losses was made.

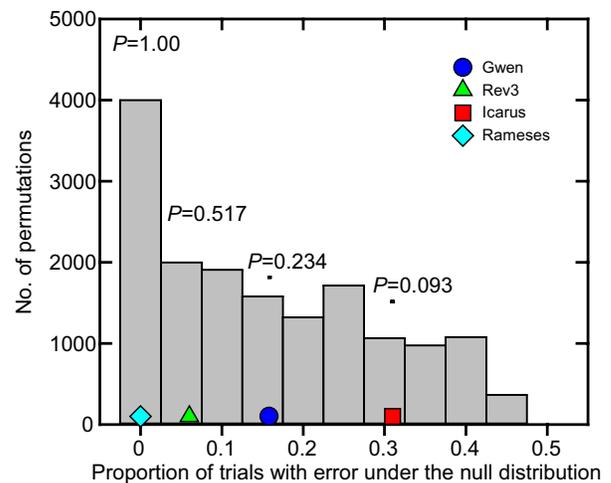
### Statistical analyses

Statistical tests were performed using SAS Statistical Software v. 9.4 (SAS Institute, Inc., Cary, NC, USA). We compared the proportions of unsuccessful flights at pre-exposure with those at 20 min, 24 h and 48 h post-exposure using a permutation test (Ernst, 2004), a method for empirically calculating the distribution of the data under the null hypothesis (no effects of noise exposure on flight performance) through repeated re-sampling of the data. The null hypothesis assumes that the number of errors made by a bat on any post-exposure day is interchangeable with the number of errors made by that bat on the pre-exposure day. That is, the only differences in the number of errors at any two time points are assumed to be due to random fluctuations in the bat's performance and not attributable to the exposure. Assuming no confounding, systematic differences in experimental conditions related to the outcome on any given test day unrelated to the noise exposure, we can construct the empirical null distribution by re-sampling the data 1000 times with the bat's error proportions randomly shuffled between pre-exposure and post-exposure. By comparing the observed error proportions to this permutation distribution, we can directly evaluate whether the assumptions of the null hypothesis hold. If the null hypothesis is not true, then the randomly shuffled data will look different from the real data. Differences in the numbers of pulses, in IPIs and in sonar sound groups across test days were assessed using separate GLM repeated measures analysis of variance (RM-ANOVA), with test day as the repeated measure. Two-sample Kolmogorov–Smirnov tests were used to compare changes in IPIs, changes in post-IPI/pre-IPI ratios and changes in relative amplitudes of pulses across test days relative to noise exposure.

## RESULTS

### Bats flew successfully through the chain array before and after noise exposure

On each of the four test days, each bat completed between 12 and 17 successful flights (Table 1). Error proportions (the proportion of unsuccessful to the sum of unsuccessful plus successful flights) at pre-exposure varied from 0 to 0.21 across the four bats. These variations indicate that the task was more difficult for some bats than for others. A permutation test was used to evaluate the effects of noise exposure on flight performance. At 20 min post-exposure, the observed error proportions are consistent with the permutation distribution (Fig. 3). That is, the distribution generated by our assumptions under the null distribution produces a range of error proportions that conforms to the observed results with a high probability. The *P*-value for each observed proportion is calculated as the percentage of the null distribution that is greater than or equal to the observed proportion. The lack of clear discordance between the observed and permutation distributions acts as evidence against rejection of the null hypothesis; i.e. it is indicative of a lack of an



**Fig. 3. Results of the permutation test.** The observed error proportions for each bat at 20 min post-exposure are plotted in relation to the empirical null distribution (x-axis). The distribution of error proportions under the null hypothesis was generated by randomly resampling the entire data set (four bats on four test days) 1000 times, resulting in 16,000 permutations (y-axis, gray bars). Empirical *P*-values of error proportions for each of the four bats all lie within the null distribution.

exposure effect. Similarly, none of the individual observed error proportions were significantly different from a null distribution at either 24 h or 48 h after noise exposure (Table S1).

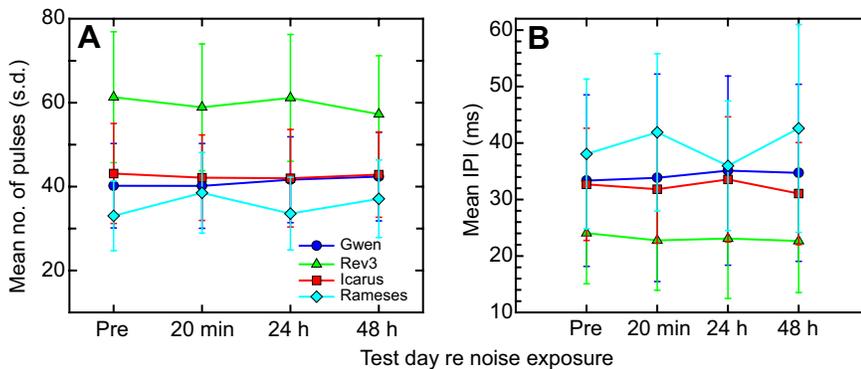
At 20 min post-exposure, only the data from one bat, Bat Icarus, even approached statistical significance ( $P=0.093$ ). Four of the six errors made by this bat occurred in the first four flight trials. There are two possible explanations for this finding. One is that Bat Icarus suffered some perceptual difficulty after the noise exposure that recovered quickly. Another possibility is that these initial errors reflect a motivational effect – the bat may have been unwilling to fly after the exposure.

### Number of emitted pulses was not affected by noise exposure

Fig. 4A shows the mean number of pulses emitted by each bat during successful flights on each test day relative to noise exposure. Individual bats differed in mean number of pulses (from 35.6 for Bat Rameses to 59.6 for Bat Rev3 averaged across all test days), suggesting that each bat used its own sampling rate to maneuver through the chain array. Results of RM-ANOVA showed a significant difference between bats in number of pulses ( $F_{3,50}=791.21$ ,  $P<0.001$ ), but no significant effect of test day (Pillai's  $V=0.014$ ;  $P=0.88$ ). There was a significant bat by time interaction (Pillai's  $V=0.619$ ;  $F_{9,150}=4.34$ ,  $P<0.001$ ), driven by the data from Bat Rameses, who varied his number of emissions from the pre-exposure level on each post-exposure day. This bat was the only one who increased emissions at 20 min post-exposure, the time point at which the effects of noise were expected to occur. But, there was no consistent trend across all bats to change the number of pulse emissions in a particular direction (increasing or decreasing) after noise exposure. The number of pulses emitted by each bat during each successful flight on each test day is plotted in Fig. S1.

### Temporal patterning of pulses was not affected by noise exposure

An increase in difficulty in navigating the flight corridor due to any possible hearing impairment caused by noise exposure may



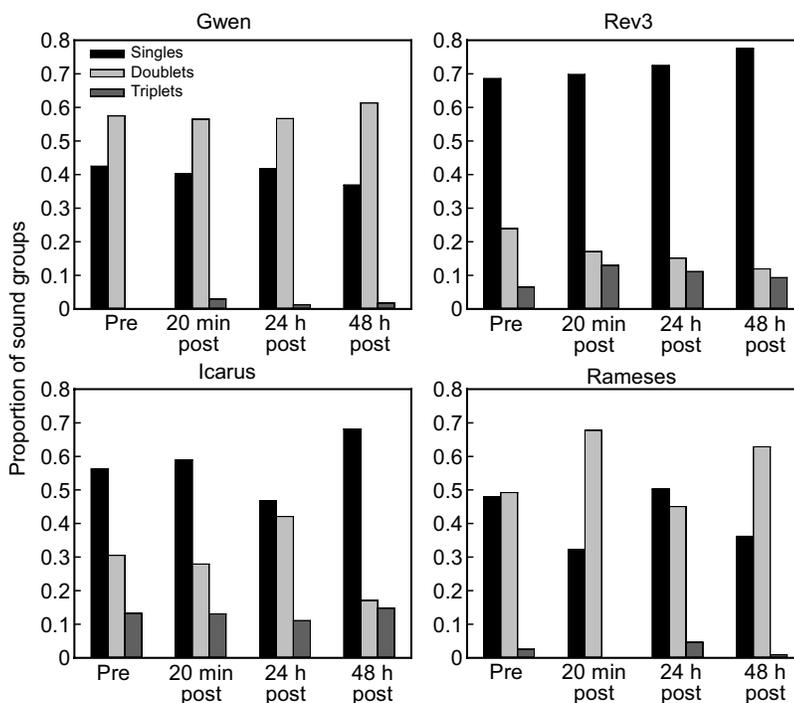
**Fig. 4. Pulse numbers and IPIs on test days relative to noise exposure.** (A) Mean number of pulses per flight ( $\pm 1$  s.d.) on successful flights for each test day for each of the four bats (as indicated by different symbols). Mean pulse number did not differ significantly across test days. (B) Mean IPIs ( $\pm 1$  s.d.) on successful flights for each test day for each bat (symbols as in A). Mean IPIs did not differ significantly across all test days. For both metrics, there are individual differences between bats both in mean values and in changes in values across test days.

manifest itself as a change in mean IPI values, the grouping of pulses into sonar sound groups or the patterning of IPIs. The mean IPIs for each bat on each test day relative to noise exposure are plotted in Fig. 4B. Individual bats differed in mean IPI, over a range from 23.1 ms (Bat Rev3) to 39.6 ms (Bat Rameses). Results of RM-ANOVA showed a significant difference between bats ( $F_{3,2403}=774.1$ ,  $P<0.001$ ), but no significant effect of test day (Pillai's  $V=0.003$ ,  $P=0.11$ ). There was a significant bat by time interaction (Pillai's  $V=0.044$ ,  $F_{3,7209}=11.89$ ,  $P<0.001$ ). At 20 min post-exposure, Bats Rameses and Gwen increased mean IPI (by 5 ms and 1 ms, respectively), whereas Bats Icarus and Rev3 decreased mean IPI (by 1 and 2 ms, respectively). Bat Rameses decreased and then increased IPI at 24 and 48 h post-exposure, whereas the other bats showed no or very small changes. Overall, there was no consistent trend across all four bats to vary IPI in a particular direction after noise exposure.

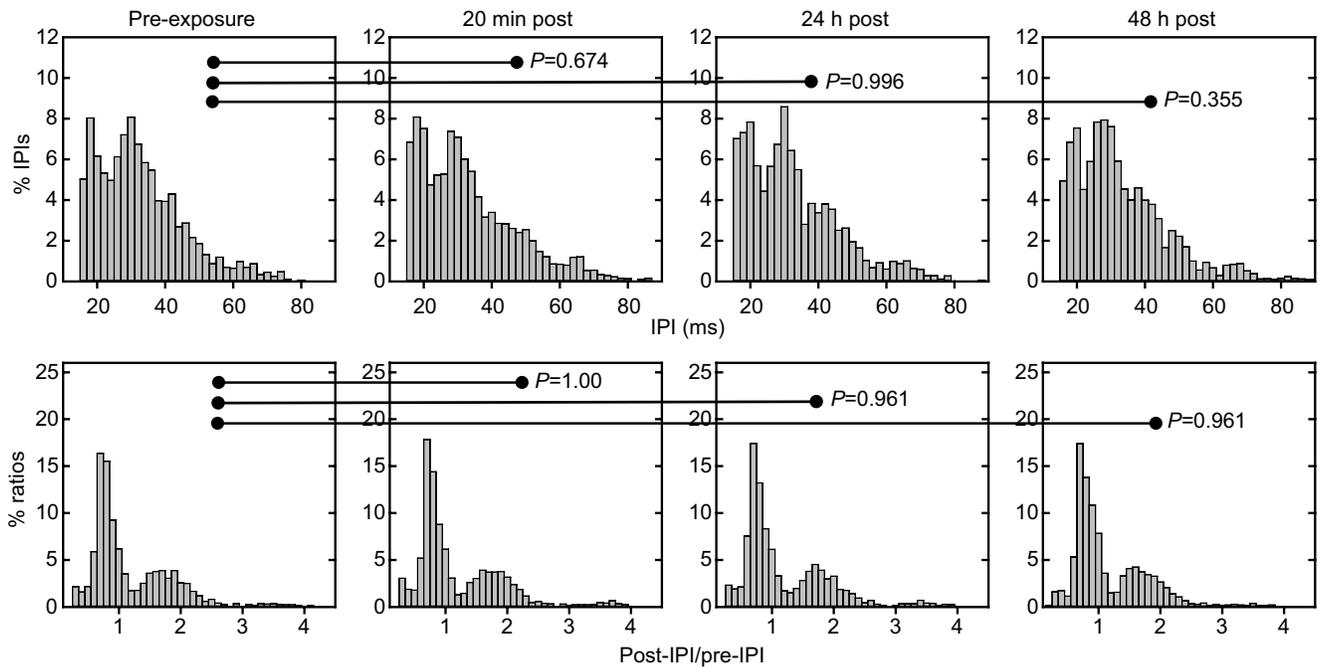
Fig. 5 shows the proportion of the different types of sonar sound groups emitted by each bat on each test day. For all bats on all test days, the largest proportions of sound groups are singles and doublets, with fewer triplets and few-to-no quadruplets. Results of RM-ANOVA show that the different types of sound groups were not equally probable ( $F_{3,9}=7.67$ ,  $P=0.0075$ ). Bats Rev3 and Icarus

emitted more singles than doublets, Bat Gwen emitted more doublets than singles, and Bat Rameses emitted different proportions of singles and doublets on the four test days. These differences between individual bats were not statistically significant ( $F_{3,9}=0.61$ ,  $P=0.62$ ). Moreover, there were no differences in the relative proportions of different types of sound groups before and after noise exposure (Pillai's  $V=0.161$ ;  $P=0.72$ ).

The patterning of IPIs (Fig. 6) is illustrated by histograms showing: (1) the distribution of percent IPIs at each IPI value (top row of plots) and (2) the distribution of percent ratios of post-IPI/pre-IPI values (bottom row of plots) on each test day relative to noise exposure (columns). These data are averaged across all bats; histograms for each individual bat are shown in Fig. S2. Histograms (2 ms bins) of percent IPIs consistently show two peaks (18 ms and 30 ms at pre-exposure; 18 and 28 ms at 20 min post-exposure; 20 ms and 30 ms at 24 h post-exposure; 20 ms and 28 ms at 48 h post-exposure). The presence of two peaks reflects the alternation of IPIs from short to long and vice versa. Results of Kolmogorov–Smirnov tests comparing each IPI distribution after noise exposure to that observed at pre-exposure indicate that these distributions do not differ statistically ( $P$ -values shown in Fig. 6, top row). The post-IPI/pre-IPI ratio histograms are segregated into bins of 0.1 ratio



**Fig. 5. Proportion of the different types of sonar sound groups, as classified using the criteria of Kothari et al. (2014), emitted by each of the four bats on each test day relative to noise exposure.** All bats emitted pulses primarily as singles or doublets, with fewer triplets. Proportion of quadruplets is not plotted because of small numbers (proportions are at or close to 0 for different bats). The proportions of the different sound groups did not differ significantly as a result of noise exposure.



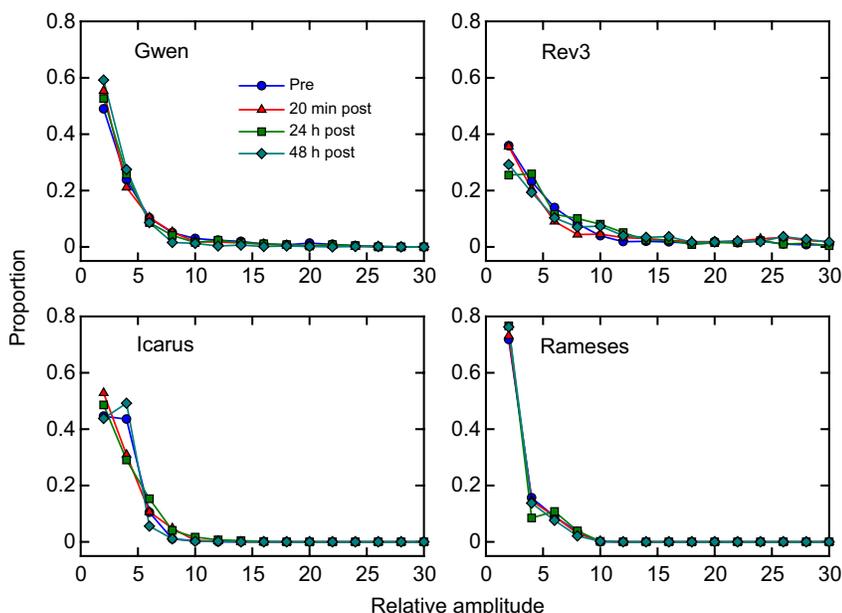
**Fig. 6. Histograms of percent IPIs and percent ratios of post-IPIs/pre-IPIs averaged for all four bats on each test day.** The bin size for the ratios (bottom row) is 0.1. The distribution at each post-exposure test day was compared with the pre-exposure distribution using a two-sample Kolmogorov–Smirnov test, with the  $P$ -value as indicated (solid lines between circles) for each comparison. None of the  $P$ -values reached statistical significance, indicating a lack of evidence for any difference between the IPI or post-IPI/pre-IPI ratio distributions related to noise exposure. IPI distributions are skewed, with two peaks centered around 18–20 and 28–30 ms. This bimodal distribution shows the staggering of short IPIs and long IPIs.

units. These histograms portray aspects of the internal complexity in the patterning of IPIs beyond what is displayed in the distributions of percent IPIs. Ratios of 1 indicate that post-IPIs and pre-IPIs are identical. The ratio histograms clearly show a sharp peak at 0.7–0.8 and a shallower, broader peak at 1.6–1.7 on all test days, indicating the presence of contingencies of short IPIs followed by long IPIs or the reverse, regardless of the classification of these contingencies into arbitrary types of sonar sound groups. Kolmogorov–Smirnov tests comparing the distribution of ratios at 20 min, 24 h and 48 h post-exposure to the distribution at pre-exposure indicate that these

distributions do not differ statistically ( $P$ -values shown in Fig. 6, bottom row). Thus, both the IPI distributions and the post-IPI/pre-IPI ratio distributions remain very similar in their shapes across all test days, regardless of noise exposure. By these metrics, noise exposure had no influence on the temporal patterning of emitted pulses.

#### Relative amplitudes of pulses was not affected by noise exposure

Fig. 7 shows the proportion of relative amplitudes of pulses emitted by each bat on each post-exposure test day, plotted for all test days



**Fig. 7. Proportion of relative amplitudes of pulse emissions do not vary significantly after noise exposure.** Data for each of the four bats are plotted in separate panels. Amplitudes of successive pulses are normalized to the amplitudes on the pre-exposure day. There are no significant differences in relative amplitudes related to test day.

for that individual in the same panel. The shapes of the distributions indicate a large proportion of lower relative amplitudes with a steep decrease towards the higher relative amplitudes; this, however, is an artifact of the lack of compensation for spreading loss with distance. Although the distributions of relative amplitudes differed between bats, results of two-sample Kolmogorov–Smirnov tests show that these distributions did not vary significantly across test days (pre-exposure compared to 20 min post-exposure,  $P=0.99$ ; pre-exposure compared to 24 h post-exposure,  $P=1.00$ ; pre-exposure compared to 48 h post-exposure,  $P=0.94$ ).

## DISCUSSION

In this experiment, we investigated whether the echolocating big brown bat adapts its pulse emissions during navigation through a densely cluttered environment 20 min, 24 h or 48 h after being exposed to intense broadband noise. We reasoned that, if the bats' perceptual acuity for detecting echoes and guiding flight was affected by prior noise exposure, then this would be reflected in a change in the number, timing or relative amplitude of pulse emissions. The data show clearly that, across all four bats, there were no significant differences in any of these measures before and after noise exposure. It is not known whether the landing buzz, which was excluded from analysis but which has been shown to index task difficulty in Daubenton's bats (*Myotis daubentonii*; Hulgard and Ratcliffe, 2016), would be affected by noise exposure. Consistent with the lack of change in pulse emission parameters, navigation errors through the chain array did not increase after noise exposure. These results indicate that big brown bats' perceptual acuity and their ability to steer flight in a complex, acoustically cluttered scene remain intact after exposure to broadband noise with the specific parameters used in this experiment. These data confirm and extend our earlier report (Simmons et al., 2016) that big brown bats do not suffer TTS after exposure to broadband ultrasonic noise at the same sound exposure level and tested at the same recovery times. It is possible that the bats' apparent lessened susceptibility to TTS, as shown in these two different procedures, is not a general phenomenon but related to the specific exposure parameters and recovery times that were tested. For example, bats may suffer some TTS, but recover by the 20 min post-exposure time. Further research assessing the impact of these other variables is needed.

### Perceptual acuity is not affected by prior noise exposure

When FM bats fly in cluttered environments, they face two constraints: (1) they have to emit their pulses fast enough to maneuver through confined spaces or to catch moving prey at short range, and (2) they need to assess the background in order to plan for the immediately upcoming flight path (Moss and Surlykke, 2010). If two successive pulse emissions are produced at a fast rate, they will have almost identical FM waveforms, and the bat may not be able to assign each returning echo to its corresponding emission, a problem known as pulse-echo, or range, ambiguity (Petrites et al., 2009). Pulse-echo ambiguity causes confusion about which echoes are related to the corresponding sonar pulses, by creating false echo-delay estimates and thus phantom objects in the spatial scene. In a chain array, each broadcast is followed by a long sequence of echoes that stretch away in time, so that pulse-echo ambiguity would be severe. As demonstrated by Petrites et al. (2009) and Wheeler et al. (2016), big brown bats navigating through chain arrays of different densities, corridor widths and curvatures alternate the temporal patterning of their pulses between short and long IPIs to assess the depth of the entire acoustic scene. As clutter density becomes tighter and spatial extent increases, IPIs become shorter and show greater

short-IPI to long-IPI staggering. This staggering of IPIs means that real echoes arrive at consistent and predictable delays, whereas ambiguous echoes arrive at inconsistent delays that fluctuate from one pulse-echo sequence to another, thus alleviating pulse-echo ambiguity. The data from the current study confirm the short-to-long staggering of IPIs in a difficult navigation task – flying through a narrow, curved, high-density corridor. Moreover, staggering of IPIs, as shown by the bimodal peaks in the IPI ratio distributions, did not change significantly 20 min after big brown bats were exposed to intense broadband noise, indicating that they remained able to solve the pulse-echo ambiguity problem.

Along with short-to-long staggering, the absolute lengths of IPIs vary with the acoustic complexity of the task the bat is asked to solve. Big brown bats flying in open space emit pulses with IPIs of around 175 ms, whereas bats flying in the laboratory near or through clutter emit pulses with IPIs ranging from 29 to 100 ms in different experimental conditions (Petrites et al., 2009; Kothari et al., 2014; Warnecke et al., 2016; Wheeler et al., 2016). Bats in our experiment flew down a narrow (40 cm) curved corridor through a high-density chain array. In this condition, mean IPI on the pre-exposure test day was 32.0 ms. Such a short IPI indicates that the flight conditions were challenging for the bat, and that performance would be expected to show strong effects of noise exposure, if any existed. Mean IPI remained stable at 20 min, 24 h and 48 h post-exposure, suggesting that the perceived difficulty of the task to the bat did not change at these time points. Still, there were individual differences between bats in both their pre-exposure and post-exposure IPIs. These individual differences indicate that bats had unique strategies for solving the navigation task, but that they largely maintained these strategies after noise exposure.

The types and proportions of sonar sound groups emitted by big brown bats also differ depending on experimental conditions and task difficulty (Moss et al., 2006; Petrites et al., 2009; Kothari et al., 2014; Sändig et al., 2014; Knowles et al., 2015; Warnecke et al., 2016), with more groups (pulses in doublets and triplets) in more difficult tasks. Navigating through a narrow corridor surrounded by high-density clutter would be expected to result in more doublets and triplets rather than singles. In such a task, Wheeler et al. (2016) reported that, as corridor width became narrower, singles and triplets increased, whereas doublets decreased. In the current study, bats emitted, on average, more singles and doublets than triplets, even though individual bats used different proportions of singles, doublets and triplets to solve the navigation task. Comparisons of types of sonar sound groups can be difficult owing to the different criteria used in different studies to define these groups (Petrites et al., 2009; Sändig et al., 2014; Knowles et al., 2015; Wheeler et al., 2016). The criteria proposed by Kothari et al. (2014), and used by Wheeler et al. (2016) and in the current study, are arbitrary, so that the classifications of pulse groups will change as 'stability' and 'island' criteria change. The metric introduced by Wheeler et al. (2016) is based on the ratio of the distributions of pre-IPI to post-IPI and do not rely on arbitrary criteria. This metric may be more suitable to comparisons across different studies.

### Effects of noise on echolocation performance

The effects of noise on pulse emissions and echolocation performance have been examined in big brown bats (Simmons et al., 1978) as well as in other FM bat species (Griffin et al., 1963; Amichai et al., 2015 and references therein), but in a different experimental paradigm – bats were tested during exposure to a simultaneous background of interfering noise, rather than after the end of noise exposure as was done here. Our design was chosen to

explicitly mimic those used to study noise-induced hearing loss in humans and other mammals (Ward et al., 1958; Finneran, 2015), and so utilized higher sound exposure levels than used in experiments examining performance during noise exposure. Our results do not imply that there would be no changes in pulse emissions or navigation errors if bats had been required to navigate the chain array while being exposed to intense noise, only that there are no after-effects of these exposures.

The lack of impairment in navigation and the absence of changes in number or temporal patterning of pulse emissions before and after intense broadband noise exposure are consistent with previous results reporting no TTS in big brown bats (Simmons et al., 2016) in either active or passive hearing procedures or in another FM bat, *Pipistrellus abramus*, using electrophysiological measures (Simmons et al., 2015). In marked contrast, the passive hearing of terrestrial mammals can be severely impaired after exposure to broadband noise of even lower sound exposure levels (Ward et al., 1958; Mills et al., 1981; Shone et al., 1991; Nielsen, 1982). We hypothesize that the special demands of echolocation have contributed to the evolution of lessened susceptibility to noise-induced hearing losses. As support, echolocating marine mammals do not show significant TTS after exposure to high-frequency noise at the levels used in this experiment, although they will show increases in hearing thresholds after exposure to noise in the low-frequency anthropogenic range (Finneran, 2015). Our data do not address the issue of whether big brown bats would suffer any TTS after exposure to low-frequency noise outside of the spectral range used for echolocation. Further experiments testing the impact of different levels, bandwidths and duration of noise in different detection and discrimination tasks are needed to assess the limits of the big brown bat's ability to reject noise interference.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

K.N.H., J.A.S. and A.M.S. designed research; K.N.H. and M.L. conducted research; K.N.H., M.L. and J.A.S. analyzed data; A.M.S. and K.N.H. wrote the manuscript.

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#### Data availability

The data from this study are publicly available at the Brown Data Repository <https://doi.org/10.73011/Z0BP00QG>.

#### Supplementary information

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