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RESEARCH ARTICLE

Adaptive changes in echolocation sounds by *Pipistrellus abramus* in response to artificial jamming sounds

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ABSTRACT

The echolocation behavior of *Pipistrellus abramus* during exposure to artificial jamming sounds during flight was investigated. Echolocation pulses emitted by the bats were recorded using a telemetry microphone mounted on the bats' backs, and their adaptation based on acoustic characteristics of emitted pulses was assessed in terms of jamming-avoidance responses (JARs). In experiment 1, frequency-modulated jamming sounds (3 ms duration) mimicking echolocation pulses of *P. abramus* were prepared. All bats showed significant increases in the terminal frequency of the frequency-modulated pulse by an average of 2.1–4.5 kHz when the terminal frequency of the jamming sounds was lower than the bats' own pulses. This frequency shift was not observed using jamming frequencies that overlapped with or were higher than the bats' own pulses. These findings suggest that JARs in *P. abramus* are sensitive to the terminal frequency of jamming pulses and that the bats' response pattern was dependent on the slight difference in stimulus frequency. In experiment 2, when bats were repeatedly exposed to a band-limited noise of 70 ms duration, the bats in flight more frequently emitted pulses during silent periods between jamming sounds, suggesting that the bats could actively change the timing of pulse emissions, even during flight, to avoid temporal overlap with jamming sounds. Our findings demonstrate that bats could adjust their vocalized frequency and emission timing during flight in response to acoustic jamming stimuli.

KEY WORDS: Jamming avoidance, Terminal frequency, Emission timing, FM bats

INTRODUCTION

Echolocating bats emit ultrasound pulses and monitor the echoes to locate insect prey and to avoid obstacles (Griffin, 1958). In the presence of conspecifics during echolocation, bats may need to employ a jamming-avoidance response (JAR) when sounds from neighboring bats cause acoustic interference. Bats are thought to have adapted to overcome interference by unexpected sounds broadcast by conspecifics. Many studies have explored JAR by bats (e.g. Habersetzer, 1981; Surlykke and Moss, 2000; Ulanovsky et al., 2004); however, the means by which bats adapt their echolocation sounds, i.e. how they actively change the characteristics of their emitted signals to minimize interference from jamming sounds close to or overlapping with their own returning echoes in time and/or frequency range, remains unknown.

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When foraging with conspecifics in the field, some frequency-modulated (FM) bat species have been observed to shift the terminal frequency portion of the downward FM sweep of the fundamental component (terminal frequency; TF) of their own pulse to maintain frequency separation between individuals, suggesting that the bats perform the JAR by shifting frequency ranges during group flight (Bartonička et al., 2007; Chiu et al., 2009; Ibáñez et al., 2004; Miller and Degn, 1981; Necknig and Zahn, 2011; Surlykke and Moss, 2000; Ulanovsky et al., 2004). JAR behavior has also been observed by generating acoustic stimuli through an ultrasonic speaker. When playback stimuli consisting of recorded echolocation sounds were presented to bats (*Tadarida brasiliensis*) during foraging in the field, the bats shifted their call frequency away from the playback frequency (Gillam et al., 2007). Previous studies have also demonstrated that stationary bats actively altered both their call frequency away from the frequency of the jamming stimulus (Bates et al., 2008; Masters et al., 1991; Tressler and Smotherman, 2009) and the timing of their pulse emission to reduce overlap between pulse and sound stimuli (Jarvis et al., 2010; Jarvis et al., 2013). These JAR studies using artificial playback jamming sounds could quantitatively characterize vocal responses of the bats, especially under stationary conditions. However, because Doppler-induced error makes it difficult to obtain accurate measurements of echolocation pulses (such as call frequency) from bats during flight, it remains unknown whether flying bats exhibit JAR by changing their acoustic characteristics, i.e. the frequency of their echolocation pulses, in the presence of sounds that overlap with the frequency range of their own sounds (similar to electric fish that shift the frequency of their electric organ discharges to increase the difference in frequency between themselves and the jamming stimulus) (Watanabe and Takeda, 1963).

In this study, the echolocation behavior of FM bats, *Pipistrellus abramus* (Temminck 1840), was investigated while the bats were exposed to artificial jamming sounds during flight in an experimental chamber. Echolocation pulses emitted by the bats were recorded using the Telemike mounted on the bat's back, and we assessed whether the bats engaged in JAR, adaptively altering the acoustic characteristics of the pulses and/or emission timing during flight.

RESULTS
Experiment 1: effect of FM jamming sounds

During the flight sessions, bats showed similar flight behaviors: flying in circles in the chamber with or without jamming sounds. The bats did not show any evasive action, i.e. flying away from the loudspeakers located at each corner of the chamber, during exposure to jamming sounds. In nine of the total of 14 jamming flight sessions, the number of pulse emissions was increased by 18 ± 11 pulses during 6 s recording, while four jamming flight sessions showed decreases by 14 ± 9 pulses compared with jamming-off
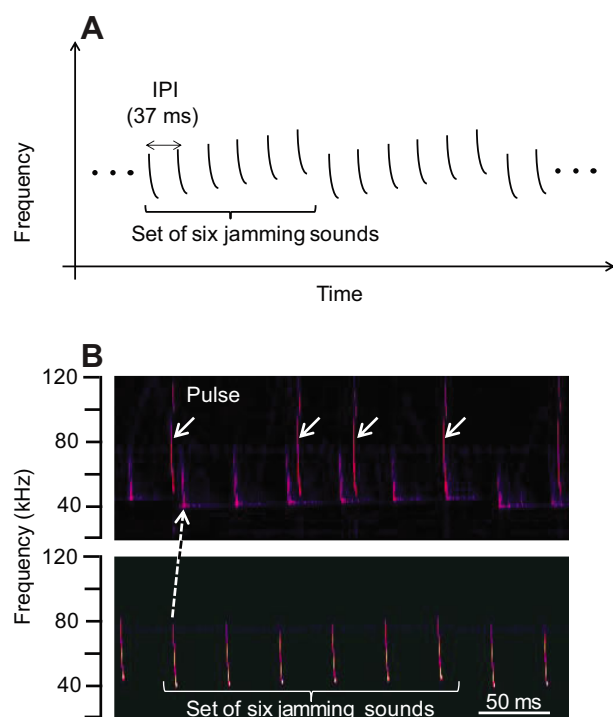
Fig. 1. Sequence of jamming sounds uses in experiment 1. (A) Schematic spectrogram of the sequence of jamming frequency-modulated (FM) sounds. The duration of each FM sound was 3 ms, including the 0.3 ms rise–fall time. The sequence consisted of six FM sounds with different frequencies and was repeatedly presented in ascending order of TFs (see Results). Two sets of sound sequences were prepared: low-terminal frequency (TF, from 39 to 44 kHz) and high-TF (from 43 to 48 kHz) series. The interpulse interval (IPI) between sounds was set at 37 ms. (B) Representative spectrograms of the sounds recorded using the Telemike (top panel) and the input electric signals (jamming sounds) of the loudspeakers (bottom panel) while the bat was flying in the chamber.

conditions, and in two sessions pulse emissions did not change. Statistically, the pulse emission rate during jamming did not differ from that during jamming-off conditions (Mann–Whitney U-test, P=0.08 low-TF series, P=0.398 high-TF series).

Two sets of six jamming FM sounds in the low and high TF series in order of TF were prepared (low TF series: 79–39, 80–40, 81–41, 82–42, 83–43 and 84–44 kHz; high TF series: 83–43, 84–44, 85–45, 86–46, 87–47 and 88–48 kHz; see Materials and methods). The interpulse interval (IPI) between sounds in each TF series was set at 37 ms, and either the low- or high-TF series was repeated during jamming (Fig. 1A). Fig. 1B shows representative spectrograms of the sounds recorded by the Telemike (top panel) and the input electric signals (jamming sounds) of the loudspeakers (bottom panel) while the bat was flying in the chamber. The jamming sounds from the loudspeakers were recorded using the Telemike on the back of the bat, indicating that acoustic jamming conditions were created.

Fig. 2A shows differences in TFs of emitted pulses with and without jamming FM sounds for the low-TF series in seven individual bats. All seven bats significantly increased the TF of the pulses during exposure to jamming FM sounds compared with the first and second jamming-off conditions (Mann–Whitney U-test, P<0.001). The mean of the increase in TF ranged from 2.1 to 4.5 kHz among the seven bats. In contrast, when exposed to jamming sounds in the high-TF series, no consistent increase in the TF was observed (Fig. 2B). These finding suggest that the bats
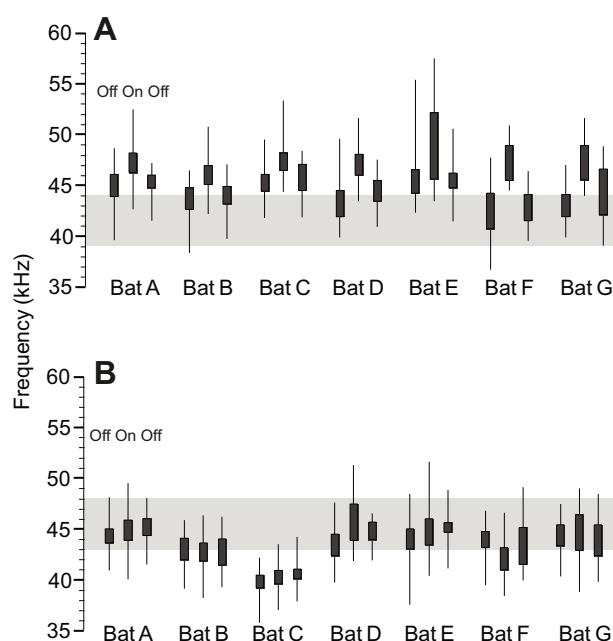

Fig. 2. Changes in TF with and without jamming FM sounds. Results are shown for low-TF (A) and high-TF (B) series from seven individual bats. The box represents the s.d. of the data; the whiskers indicate the minimum and maximum. The shaded area indicates the frequency range of TFs of FM jamming sounds in each TF series. In the low-TF series, all seven bats significantly increased the TFs of the pulses under jamming-on conditions (Mann–Whitney U-test, P<0.001).

shifted their own TFs in response to FM jamming sounds with TFs lower than their own TFs.

Fig. 3A,B compares the distributions of pulse duration between jamming-on and -off conditions. The pulse duration was normalized based on the mean of the pulse duration emitted under jamming-off conditions. For both low- and high-TF series, all seven bats extended their pulse duration during exposure to jamming sounds (Mann–Whitney U-test, P<0.001, low-TF series: 1.6±0.6 ms for jamming-off and 2.0±0.6 ms for jamming-on; high-TF series: 1.7±0.5 ms for jamming off and 2.1±0.7 ms for jamming on). Fig. 3C shows representative spectrograms of emitted pulses under jamming-off and -on conditions. Under jamming conditions, the bats lengthened the duration of the end-frequency portion of the downward FM sweep, emphasizing the TF. In addition to extending the pulse duration, the bats in flight emitted pulses of slightly greater intensity. The means of changes in the sound pressure levels of emitted pulses during jamming-on conditions compared with initial jamming-off conditions ranged from −3 to +8 dB (low-TF condition: 2.6±3.1 dB, high-TF condition: 2.7±2.6 dB), with a mean of +2.5 dB throughout all flight sessions.

Experiment 2: effect of band-limited noise
Pulse emission timing
To assess whether the bats changed pulse emission timing to temporally avoid jamming sounds during flight, three band-limited noise sounds with different frequency bands and one constant frequency (CF) tone of 70 ms duration were prepared in experiment 2 (see Materials and methods and Fig. 5A). Fig. 4 shows spectrograms of the sounds recorded using the Telemike (top panel) and the input signal of jamming sounds (bottom panel), which suggest that the bats adjusted pulse emission timing to avoid temporal overlap with jamming sounds. For quantitative assessment,
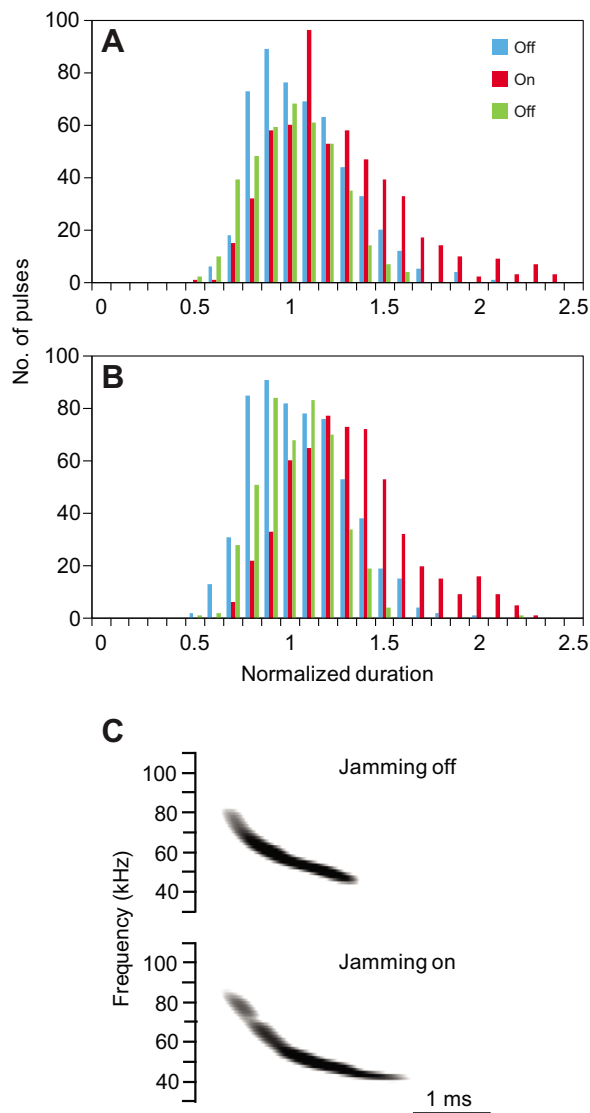
Fig. 3. Comparison of distributions of pulse duration between jamming-on and -off conditions. Results are shown for the low-TF (A) and high-TF (B) series. Data were taken from all flight sessions of seven bats. The pulse duration was normalized based on the mean duration of pulses emitted under jamming-off conditions. Pulse durations were significantly increased during jamming-on conditions for both low- and high-TF series (Mann–Whitney *U*-test, *P*<0.001). (C) Spectrograms of representative echolocation pulses emitted without (top) and with (bottom) jamming sounds.

we compared the probability of pulse emission during the silent period with and without (control) jamming sounds (note that the probability of pulse emission under control conditions was calculated using the number of pulse emissions during the silent period when the loudspeakers were turned off). We found that in 84% of flight sessions (21/25 flights), the probability of pulse emissions during the silent period under jamming conditions was higher than that under control conditions (Fig. 5B). As there was no significant change in the number of pulse emissions during the noise period between flights with and without jamming sounds (Mann–Whitney *U*-test, *P*=0.242), the bats emitted pulses more frequently during the silent period when they were exposed to jamming sounds (Fig. 5B). Fig. 5B also shows that pulse emission was adjusted regardless of differences in the frequency width of jamming sounds [a: 100% (9/9), b: 83% (5/6), c: 67% (4/6), d: 75% (3/4)].

TF and duration

The TF slightly increased under jamming conditions compared with control conditions (Fig. 6A). The mean TF shift ranged from 0.4 to 1.5 kHz among four types of jamming sounds. Only 8% of the cases (2/25 flight) showed a significant decrease in TF under jamming conditions (Mann–Whitney *U*-test, *P*<0.001).

In contrast, Fig. 6B shows changes in pulse duration for four jamming sounds. The pulse duration did not show any consistent trend throughout the flight sessions (increase with jamming on compared with jamming off, three flights; decrease, one flight; or no change, 21 flights: Mann–Whitney *U*-test, *P*<0.001).

DISCUSSION
Shift in TF under jamming conditions

Previous JAR studies have suggested that FM bats actively alter their TF to avoid overlap with the frequency of jamming stimuli such as artificial playback jamming sounds and sounds from neighboring conspecific bats. For example, JARs by shifting the TF away from the frequency of the jamming stimulus were characterized quantitatively for stationary bats based on acoustic measurements of pulses emitted during target-detection tasks (a two-alternative forced-choice experiment) (Bates et al., 2008; Masters et al., 1991) or during crawling in the cage (Tressler and Smotherman, 2009). However, in the JAR studies using bats during flight, it is difficult to determine whether bats significantly change the acoustic characteristics of emitted pulses because it is difficult to measure acoustics from flying bats under controlled experimental conditions. In this study, using an on-board telemetry microphone, slight changes in the frequency of pulses emitted by flying bats could be measured without Doppler error induced by flight. As a result, we found that, during flight, the bats significantly increased their TFs during exposure to FM jamming sounds, which is indicative of adaptive frequency shifts by FM bats to avoid acoustic jamming during flight.

Fig. 2A shows that all bats used in the experiment consistently increased their TFs in response to low-TF jamming FM sounds. These findings suggest that when the TFs of jamming sounds were lower than the bats' own TFs, bats shifted the TF of their emitted pulse to increase the frequency difference between the bats' pulse and jamming FM sounds. Interestingly, as shown in Fig. 2B, no clear tendency toward changes in the bats' TFs was observed in the high-TF series in which the TFs of jamming sounds overlapped or increased compared with the frequency range of the bats' own TFs. (Note that we determined the frequency emitted from the loudspeakers without any correction for Doppler shift induced by the bat's flight because the estimated Doppler shift was less than 0.3–0.5 kHz.) These results suggested that the JAR in *P. abramus* was sensitive to the TF of the pulses, and the response pattern of the bats appeared to be dependent on the slight difference in stimulus frequency.

For experiment 2, the amount of TF shift tended to increase with decreased frequency width of band-limited noise, and a CF tone at the TF of individual bats resulted in the greatest frequency shift in the TFs in echolocation pulses among the four types of jamming sounds (Fig. 6A). Further investigations are required to explore the detailed relationship between the difference in stimulus frequency and bat responses.

Pulse emission timing

Several studies have explored adjustments of pulse emission timing by bats when exposed to jamming sounds or neighboring bats flying together during echolocation (Chiu et al., 2008; Jarvis et al., 2010;
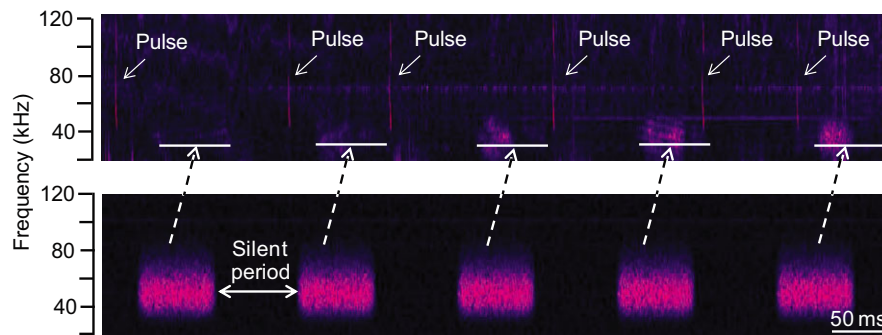
Fig. 4. Spectrograms of the sounds recorded using the Telemike (top panel) and input electric signal of band-limited jamming sounds (bottom panel, ±5 kHz bandwidth centering TF with 70 ms duration shown in Fig. 5A) in experiment 2. In this case, each sound was repeatedly presented with 140 ms IPI (noise:silence 1:1), and the silence period was 70 ms. Bar in the top panel indicates the jamming sound reaching the bats.

Jarvis et al., 2013; Obrist, 1995). For example, when bats in flight were echolocating with other conspecifics, the bats were observed to suppress their pulse emissions (Chiu et al., 2008; Obrist, 1995). Furthermore, when bats (*T. brasiliensis*) placed in a cage were exposed to jamming sounds that mimicked the bats' own echolocation pulse or noise burst, the emission rate significantly decreased (Jarvis et al., 2013) or the timing of pulse emission was altered to avoid temporal overlap with noise bursts (Jarvis et al., 2010). These JAR studies using playback stimuli quantitatively characterized acoustic responses of the bats in terms of pulse emission timing in response to jamming sounds rather than in terms of acoustic measurements from bats during group flight with conspecifics. In the present study, we exposed the bats to band-limited noise during flight in the chamber. In addition, by correcting for the sound traveling times based on the 3D coordinate data of the bats and the Telemike recordings, the time at which the bats received the jamming sound was determined precisely, which first allowed us to evaluate the time course of the JAR responses of the bats during flight. As a result, we found that bats during flight shifted the timing of their own pulse emissions in response to repeated artificial jamming sounds, and frequently produced the pulse during the silence between jamming sounds. In general, bats are known to change pulse emission timing depending on the distance to a target. Similarly, during exposure to jamming sounds, bats are thought to adjust their emission timing both to avoid temporal overlap with jamming sounds and to avoid collision with the walls of the chamber. This required much more complicated and advanced echolocation compared with that required of bats in stationary JAR studies. In future studies, it will be necessary to address these two behavioral responses separately, and interaction between JAR and flight-induced echolocation behavior will provide new insight into bat research.

In this study, we did not observe any obvious behavioral differences in the adjustment of pulse emission timing among four types of jamming sounds, which had the same signal duration but different frequency ranges in experiment 2, suggesting that adjustment of pulse emission timing is consistent during JAR if there is a possibility of temporal overlap with jamming sounds.
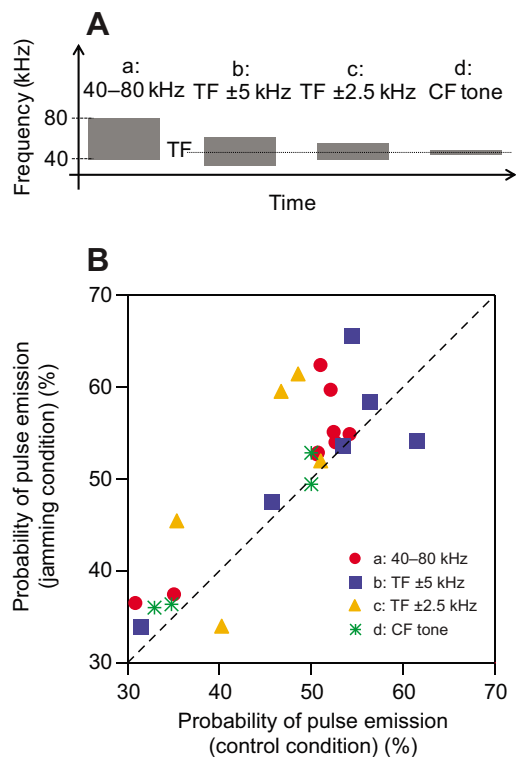

Fig. 5. Effect of band-limited noise on pulse emission timing.
(A) Schematic spectrograms of jamming sounds used in experiment 2 [a: band-limited sounds from 40 to 80 kHz, b: ±5 kHz bandwidth centering TF, c: ±2.5 kHz bandwidth centering TF, d: constant frequency (CF) sound at the TF of each bat]. (B) Comparison of the probability of pulse emission during the silent period with and without (control) jamming sounds. Data were taken from all flight sessions using eight bats (25 flight sessions). Dashed line indicates no change in the probability of pulse emission between jamming and control conditions.
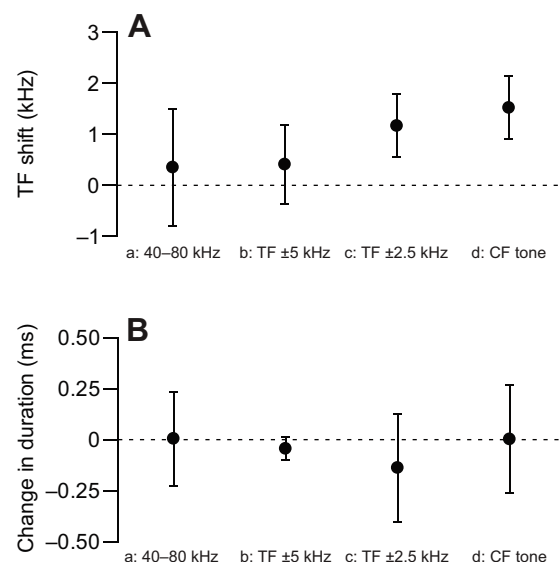

Fig. 6. Comparisons of TF shift and extension of pulse duration during jamming-on conditions and control conditions among four type of jamming sounds. (A) TF shift. (B) Change in duration. Data were taken from all flight sessions of eight bats.

Duration and sound pressure level of emitted pulses

In the presence of broad-band noise, the bats emitted more intense and longer duration pulses, sometimes accompanied by a pitch shift in vocalization (Hage et al., 2012; Schmidt and Joermann, 1986; Simmons et al., 1978; Tressler and Smotherman, 2009), which has been suggested to be a Lombard response. The increase in both the amplitude of vocalization and the duration of the pulses facilitates receiving the returning echoes in the presence of jamming sounds. In the present study, *P. abramus* significantly extended the duration of their pulses, along with a slight increase in the sound pressure level of emitted pulses, in the presence of FM jamming sounds. Interestingly, the increases in duration and sound pressure level of emitted pulses were observed in both high- and low-TF series in experiment 1, suggesting that the bats could shift the frequency of emitted pulses independently of adjustments in duration and amplitude.

Previous papers have shown that the neurons in the inferior colliculus respond selectively to particular pulse durations (duration-tuned neurons) (Casseday et al., 1994; Ehrlich et al., 1997; Fuzessery and Hall, 1999; Mora and Kössl, 2004). In terms of biological importance, neural tuning for duration is considered to be useful in enabling bats to identify their own echoes by duration. In this study, *P. abramus* lengthened their pulse duration responses to jamming FM sounds mimicking the echolocation pulse. Such adjustments in pulse duration may represent active JAR responses by bats, depending on their surrounding acoustic conditions.

However, significant extension of pulse duration was not observed in experiment 2. This may be because of differences in sound pressure levels of the jamming sounds between experiments 1 (93–95 dB SPL) and 2 (80 dB SPL). Additionally, two loudspeakers were used in experiment 2, whereas four loudspeakers were used in experiment 1, which caused differences in the acoustic field in the chamber. As we hypothesized that the degree of increase in sound pressure level and extension of duration of the emitted pulse were associated with the intensity and frequency range of jamming sounds, further investigations are required.

During echolocation in response to acoustic jamming stimuli, *P. abramus* use FM pulses that are flexible in terms of frequency structure and pulse emission timing. However, interactions between the spectral and temporal JAR of bats during flight remain unclear. Currently, JAR represents an unresolved question in studies on echolocating bats, and the investigation of complex JARs under flight-induced echolocation tasks will provide a new perspective on active sensing behavior by animals, and will have applications in engineering.

MATERIALS AND METHODS
Subjects

A total of 14 adult Japanese house bats (*P. abramus*, body length 4–6 cm, body mass 5–10 g) were used (three male, 11 female). The bats were captured from a large colony roosting in bridge girders near the campus of Doshisha University, Japan, under license and in compliance with current Japanese laws. The animals were kept in a rearing cage (30×30×20 cm) in a temperature-controlled room and were allowed free access to food (mealworms) and water in the cage. Before the experiment, the bats were trained daily to fly freely for several hours in a large flight chamber, measuring 8×3×2 m (L×W×H) to maintain their strength and health. *Pipistrellus abramus* emit downward FM pulses with harmonics, and the frequency of the fundamental component is exponentially modulated from approximately 100 to 40 kHz. The means of the starting frequency and the TF of the FM pulse were 86.6±6.8 and 43.4±1.6 kHz for the 14 bats used in this experiment.

These experiments complied with the Principles of Animal Care, publication no. 86-23, revised 1985, of the National Institutes of Health, and with current Japanese laws. All experiments were approved by the Animal Experiment Committee at Doshisha University.

Experimental procedures
Experiment 1

Seven bats were used in experiment 1. The experiments were conducted in the flight chamber under long-wavelength lighting with filters (>650 nm) to avoid any visual effects. The flight chamber was constructed of steel plates to minimize interference from external electromagnetic noises and waves used by commercial FM radio stations. Four loudspeakers (Pioneer Corporation, PT-RT III, Kanagawa, Japan) were set 0.5 m from the floor at each corner of the chamber. The speakers provided a flat frequency response over the range 20–80 kHz within ±3 dB.

The experimental procedure was as follows. First, the experimenter released an individual bat from one end of the flight chamber, and each bat flew in the absence of jamming sounds for a few minutes. Jamming sounds were then simultaneously presented from the four loudspeakers for a few minutes. During each flight condition (initial jamming off, jamming on, and then jamming off), recordings were conducted for 6 s while the bats showed continuous stereotypical U-turn flights in the chamber. All bats could usually fly two laps of the chamber, which took 5–6 s. To record the sounds of all bats under equal conditions, as far as possible, we used a 6 s period for sound recording in this study. We assessed whether the bats altered their acoustic parameters of emitted pulses during flight in response to jamming sounds by comparing the recordings with those in the absence of jamming sounds.

In experiment 1, downward FM sounds mimicking the echolocation pulse emitted by *P. abramus* were created using Cool Edit 2000 (Syntrillium Software Corporation, Phoenix, AZ, USA). *Pipistrellus abramus* are known to use highly flexible pulse repertoires during foraging in a field, i.e. the bats often emphasize sound energy in the TF, creating a quasi-CF portion following the initial FM sweep (Hiryu et al., 2008a). To mimic these echolocation pulses, each jamming FM sound consisted of a downward FM sweep portion (2 ms duration) following the 1 ms CF portion (total duration of the sound was 3 ms, including a 0.3 ms rise–fall time). A total of 10 jamming FM sounds with different TFs were created (frequency range: 79–39, 80–40, 81–41, 82–42, 83–43, 84–44, 85–45, 86–46, 87–47 and 88–48 kHz) so that the TFs of jamming sounds covered the frequency range of TFs among seven bats used in this experiment (the average TF ranged from approximately 40 to 46 kHz in the seven bats). Using these 10 jamming sounds, two sets of six sounds in the low- and high-TF series in order of TF were prepared (low-TF series: 79–39, 80–40, 81–41, 82–42, 83–43 and 84–44 kHz; high-TF series: 83–43, 84–44, 85–45, 86–46, 87–47 and 88–48 kHz). The TF of *P. abramus* tended to vary within an individual from day to day and/or during the experiment. Therefore, two simple sets of progressively increasing TF sounds were used in this experiment, such that the TFs of the artificial FM sounds could cover part of the frequency range of bat TFs, even when these fluctuated during the experiment. The IPI between sounds in each TF series was set at 37 ms, which was half of the mean IPI (74 ms) of bats flying in circles in the chamber without jamming, so that at least one artificial FM sound could be presented between pulse emissions in almost all situations (Fig. 1A). Either the low- or high-TF series was repeated during jamming.

Electric input signals were applied to the four loudspeakers through a high-speed data-acquisition card (16 bit, fs=1 MHz; National Instruments, Model NI PXIe-1073, Tokyo, Japan) and band-pass filter (20–150 kHz: NF Corporation, Model 3625, Yokohama, Japan). The sound pressure level of the jamming FM sounds ranged from 93 to 95 dB SPL peak to peak at 1 m from the loudspeaker.

Each bat was tested using both the low- and high-TF series. A total of 50–119 pulses (mean pulse number, 75) from each bat were analyzed under jamming on/off conditions.

Experiment 2

A total of eight bats were used in experiment 2 (one of which was also used in experiment 1). In addition to the acoustic parameters of emitted pulses, we assessed whether the bats changed pulse emission timing to temporally avoid jamming sounds during flight. Two loudspeakers (Panasonic,

Technics, EAS-10TH400B, Osaka, Japan) were set 1 m from the front wall and 1.2 m from the ground in the flight chamber, with 1 m between them. Fig. 5A shows the four types of jamming sounds used in experiment 2. Three band-limited noise sounds with different frequency bands and one CF tone were prepared (a: band-limited noise sounds from 40 to 80 kHz, b: ±5 kHz bandwidth centering TF, c: ±2.5 kHz bandwidth centering TF, d: CF sound at the TF of each bat). Prior to the experiment, the mean TF was defined for each bat based on pulses recorded using the Telemike while each bat flew in the chamber in the absence of jamming sounds. White noise (for the band-limited noise) and the CF tone were created using Cool Edit 2000 (70 ms duration including a 7 ms rise–fall time, 80 dB SPL peak to peak at 1 m from the loudspeaker). Note that different loudspeakers were used in experiments 1 and 2, because more intense jamming sounds were used to introduce more complex jamming conditions in experiment 1. The digitized signals were applied simultaneously to the two loudspeakers through a high-speed data-acquisition card (16 bit, fs=1 MHz; National Instruments, Model NI PXIe-1073) and band-pass filter (NF Corporation, Model 3625) so that the frequency range of the white noise was modified to present each band-limited noise, with total signal bandwidth spanning the range shown in a–c of Fig. 5A. As the mean IPI of *P. abramus* was approximately 60–70 ms during flight in the absence of jamming sounds in the chamber, the duration of the jamming sound was set at 70 ms so that at least one emission was expected to be temporally overlapped with the jamming sound. Each type of jamming sound was repeatedly presented in either 140 ms (noise:silence 1:1) or 105 ms repetition periods (noise:silence 2:1). The timing of the bats' receiving the jamming sound was obtained after correcting for the sound traveling time from the loudspeaker to the bat, which could be calculated based on 3D coordinate data from the high-speed video cameras.

The experimenter released an individual bat from one end of the flight chamber, and each bat flew in the absence of jamming sounds for a few minutes. Jamming sounds were then simultaneously presented from the two loudspeakers for a few minutes. During each condition of the flight (jamming off and jamming on), recordings were obtained for 6 s each. In experiment 2, a total of 25 flight sessions were conducted using eight bats and four types of jamming sounds (two to five flight sessions from each bat).

Video recording

Flight behaviors of the bats were recorded using two digital high-speed video cameras (IDT Japan Inc., MotionPro X3, Tokyo, Japan) located behind the left and right corners of the flight chamber so as not to interfere with the bat's flight path. The video cameras recorded 125 frames s^{-1}, and 3D coordinates of each bat's flight path were reconstructed from the video images using motion-analysis software (Directcorporation, DIPPMotionPro ver.2.2.1.0, Tokyo, Japan). Prior to recording the bat flights, a 3D reference frame with known coordinates was positioned in the center of the flight chamber and was briefly recorded using the two video cameras. The analysis software calibrated the 3D flight path reconstruction system using the camera's stereo view of the reference frame. Based on a direct linear transformation technique from the reference frame's coordinates, successive positions of the flying bat, as well as the locations of other objects, were reconstructed from video-scene coordinates measured from the pair of 2D video images. Using the 3D coordinate data, the flight trajectory of the bat was determined in conjunction with the acoustic characteristics of the bat's echolocation sounds.

Telemike recordings

Echolocation sounds emitted from the flying bat were recorded using a custom-made telemetry microphone (Telemike) mounted on the bat. The recording procedure was described previously (Hiryu et al., 2008b). The Telemike consisted of a 1/8 in omni-directional condenser microphone (Knowles, Model FG-3329, Itasca, Illinois, USA), a miniature custom-designed FM transmitter unit, a 1.5 V hearing-aid battery (Sony, Type SR521SW, Tokyo, Japan), and a transmitting antenna. The total weight of the Telemike was approximately 0.6 g. The Telemike was attached to the back of the bat with a piece of double-sided adhesive tape. The microphone pointed forward and was positioned approximately 1 cm above the mouth in the center of the right and left pinnae of the bat. The Telemike's transmitter produced radio signals that were received by an FM antenna (RadioShack

Corporation, Model15-1859, Fort Worth, TX, USA) tethered to the ceiling of the flight chamber. The received signals were demodulated to recover the bat's ultrasonic broadcasts using a custom-made FM receiver. The total frequency response of the Telemike system was flat within ±4 dB between 20 and 100 kHz. The signals from the receiver were then band-pass filtered from 20 to 150 kHz (NF Corporation, Model 3625) and digitized using a DAT recorder (16 bit, 384 kHz, SONY, Model SIR-1000W, Tokyo, Japan) with the control signal that triggered video recordings. All digitized data were stored as files on the hard disk of a personal computer so that the sound recordings could be synchronized with flight coordinates.

Sound analysis

The acoustic characteristics (TF, duration, sound pressure level) of the pulses emitted by the bat during flight were analyzed from a spectrogram of Telemike data using custom-written MATLAB routines on a personal computer. Each pulse was extracted from the sound recordings, and the fundamental components were analyzed. Duration was determined from the spectrogram at −25 dB relative to the peak intensity of the pulse. The sound pressure level was determined based on the peak energy of each pulse (0 dB was defined as the energy maximum throughout each flight).

For statistical comparisons, the Mann–Whitney *U*-test was used to test for significant differences in call parameters between data sets.

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