Strategies for Mitigation of Signal Jamming in Bat Biosonar

A dissertation submitted for the degree of
Doctor of Philosophy

by
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November 2018

Abstract

Echolocation in bats and dolphins is known as acoustic “auto-communication” because an individual animal is both the sender and receiver of a signal. Bats listen to the echoes of their own ultrasonic vocalizations, using them to construct three-dimensional images that enable them to hunt, orient themselves, and navigate in the dark. Here, I investigated behavioral and neural adaptations in echolocating bats that enable them to improve the quality of signal transmission in the presence of noisy conspecifics.

Using a telemetry microphone system, I investigated echolocation pulses emitted by *Miniopterus fuliginosus* while exposed to various types of artificial jamming sounds. This species emits brief downward frequency-modulated (FM) echolocation sounds during flight. I found that *M. fuliginosus* shifted the terminal frequency (TF) of the FM pulses they emitted in the presence of pulse mimics with a lower TF than their own. They shifted TF in response to pulse mimics, time-reversed versions of the pulse mimics, and constant-frequency (CF) sounds, but not in response to downward or upward linear FM sounds. These results suggest that spectral contents in acoustic interference are key components enabling bats to extract the echoes of their own calls from jamming signals.

I developed a telemetry microphone system for multi-channel recording and separately measured the echolocation pulses of each individual *M. fuliginosus* flying in a group of four bats. I found that the bats used different TF channels during group flight, whereas their TFs were similar during single flight. The pulses of individual bats became more dissimilar during group flight. I found that a maximal decrease in the similarity of FM signals mimicking bat pulses could be achieved with minimal TF manipulation, making TF frequency adjustment a useful strategy for segregating echolocation sounds.

I also investigated echolocation behavior during group flight in a different species, *Rhinolophus ferrumequinum nippon*. These bats use CF-FM sounds for orientation and hunting. They control pulse frequency to stabilize echoes within a narrow frequency range to which their auditory sensitivity is extremely high; this behavior is called Doppler shift compensation. Bats were flown alone and in groups of three. In group flight, they decreased the duration of CF–FM sounds and increased the duration and bandwidth of terminal FM sounds, without any clear pattern of change in the echo frequency. For an individual bat, the frequencies of the pulses produced by other bats were broadly distributed compared to the compensated frequency of its own echoes. These results suggest that the auditory system of *R. ferrumequinum nippon*, tuned to a narrow frequency range, contributes to their ability to extract their own echoes from the sounds of other bats.

I recorded brainstem auditory evoked potentials (BAEPs) from *Pipistrellus abramus* to confirm the masking effects of stronger pulses made by other bats on weak echoes. Masking effects were reduced by increasing the interval and frequency differences of two successively presented FM sounds, suggesting that frequency shifting of jamming avoidance responses (JAR) as well as coordination of emission timing can reduce the masking effect of sounds produced by other bats.

In this dissertation, I discovered behavioral adaptations to reduce jamming in the auto-communication systems of bats, revealed by flight experiments using a highly sophisticated recording technique, computations, and electrophysiological methodology. Some of the adaptations made by echolocating bats to separate their own echoes from the sounds of noisy conspecifics may have future engineering applications for radar or sonar systems.

Acknowledgements

First, I would like to express my sincere gratitude to my advisor, Professor Yoshiaki Watanabe, for his continuous support of my doctoral research. His guidance helped me throughout my research and the writing of this thesis.

I owe an enormous debt of gratitude to Professor Shizuko Hiryu for her insightful advice and warm encouragement. Without her knowledge, expertise, and hours of dedication, I could not have done this research. Doing research with her was always challenging and fun, and stimulated great personal growth.

I also thank Associate Professor Kohta I. Kobayasi for his support over the years. His expertise and broad knowledge led me in the right direction.

Many thanks go to all of the members of the Neuroethology and Bioengineering Laboratory for their help with animal care. I couldn't have completed my research without the assistance of many collaborators. I especially want to thank Takara Miyamoto, Yukimi Kadoya, Yosuke Maitani, Yuki Takeuchi, Yuko Suzuki, and Saori Sugihara for their help, advice, and encouragement throughout this project.

I would like to thank the Japan Society for the Promotion of Science for providing me with a grant for two years.

Finally, I would like to say thank you to my parents, Masakazu and Yukari, for their warmest support and affection. I will always appreciate everything you have done for me.

Kazuma Hase

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Chapter 1: General introduction

A broad range of animal species demonstrates acoustic communication. The transmission of acoustic signals from a sender to a receiver can be impeded by background noise, including biotic noise generated by animals (H. Brumm, 2006; W. Halfwerk et al., 2015; C. Q. Stanley et al., 2016), abiotic noise from wind, rain, or flowing water (M. Penna et al., 2005), and urbanization (J. L. Blickley et al., 2012; T. Lengagne, 2008; H. Slabbekoorn, 2013). Various behavioral and neural adaptations can protect signal transmission under various types pf background noise. Understanding these adaptations is important to identify the neural mechanisms that extract information from sensory flows and to improve human communication systems such as radar or sonar.

Echolocation in bats and dolphins is acoustic “auto-communication”, as an echolocating animal is both the sender and receiver of a signal (A. J. Corcoran & C. F. Moss, 2017). Bats emit ultrasonic vocalizations through their mouths or nostrils, listen to echoes returning from surrounding objects, and reconstruct three-dimensional images to navigate in the dark. To perform the reconstruction, bats compare their original emission with returning echoes that have been changed by the surroundings. They obtain the distance of an object by measuring the time between pulse emission and echo reception (J. A. Simmons, 1971), and can localize an object using monoaural and binaural cues driven by their external ears(M. J. Wohlgemuth et al., 2016b). Echolocating bats can reconstruct 3D images of their environment from the analysis of vibration through air as a sound wave.

Bats are an ideal system for studying how acoustic signals are transmitted under background noise because their survival requires that they interpret weak acoustic signals in the presence of noisy conspecifics. They intermittently emit echolocation signals during flight, which allows us to examine the relationship between their habitat and characteristics of emitted pulses. Similarly, we can also infer their perceptual adaptations as a receiver from the spectro-temporal characteristics of the pulses they use and changes in those characteristics in the presence of noise. In this dissertation, I used bats as a model to investigate how echolocation signals are modified to improve the quality of signal transmission in an auto-communication system.

1.1. Echolocation pulse design

Echolocating bats have species-specific echolocation pulses and auditory systems that are adapted to their foraging environment (G. Neuweiler, 1984, 1990). A majority of echolocating species emit frequency-modulated (FM) ultrasonic sounds (20–150 kHz), to which bats are sensitive. Other species use constant-frequency (CF)–FM calls consisting of a long CF component flanked by upward FM (initial FM) and downward FM (terminal FM) components. By controlling the frequency of emitted pulses to cancel the frequency changes in the echoes induced by the Doppler effect, the bats compensate their echo frequency within a certain frequency range, called the "auditory fovea," where their auditory sensitivity is highest (S. Hiryu et al., 2008b). By performing this Doppler shift compensation, they can detect small insects whose fluttering causes frequency modulations within the auditory fovea (H.-U. Schnitzler & A. Denzinger, 2011).

Echolocation sounds emitted by bats are highly adaptive. When searching for small insect prey, they emit relatively long, narrow band FM pulses lasting 10 milliseconds or more to make their echoes more detectable (S. Hiryu et al., 2008a; E. K. Kalko & H.-U. Schnitzler, 1993; A. Surlykke & C. F. Moss, 2000). They direct their sonar beams toward prey, increasing the pulse emission rate and bandwidth while decreasing the pulse duration as they approach their target (A. Surlykke & C. F. Moss, 2000). Immediately before capturing the prey, their pulse emission rates increase up to 200 times per second (C. P. Elemans et al., 2011). These behaviors enable them to perform highly sophisticated behavior, e.g., capturing successive small, flying insects within one second (E. Fujioka et al., 2016; E. Fujioka et al., 2011; M. Sumiya et al., 2017).

Bat echolocation pulses vary among individuals. W. M. Masters et al. (1995) suggested that echolocation pulses of big brown bats (*Eptesicus fuscus*) convey individual information such as sex, age, and family that could be used to identify bats in social interactions or to reduce jamming effects from other bats. It is also reported that trained bats can recognize not only familiar individuals but also unknow individuals, based on their vocalizations (Y. Yovel et al., 2009).

1.2. Perceptual problems of bat echolocation

Natural environments may cause difficulties in echolocation, including the following perceptual problems that degrade the perception of faint echoes.

Auditory masking: Echoes reflected from small targets are weak because of scattering and ultrasound attenuation by the air (B. D. Lawrence & J. A. Simmons, 1982). In contrast, the pulses are much more intense than are the echoes (S. Hiryu et al., 2007). How do bats deal with this masking problem?

Clutter interference: In natural environments, all objects within a detectable range are potential sources of echoes (J. A. Simmons, 2014). How do bats segregate individual echoes from such mixtures to direct their attention to a focal object while ignoring the others?

Cocktail party nightmare: Bats are highly social and often echolocate along with many conspecifics (D. K. Dechmann et al., 2009; D. K. Dechmann et al., 2010; L. N. Kloepper & M. Kinniry, 2018; L. N. Kloepper et al., 2016). When multiple bats are flying together, sounds emitted by all individuals create complex acoustic situation, called a "cocktail party nightmare" (N. Ulanovsky & C. F. Moss, 2008). Vocalizations from conspecific bats may be similar in spectral or temporal features; how do individual bats avoid confusion with another bat's calls?

1. 2. 1. Auditory masking

Bat echolocation pulses are extremely intense, reaching a maximum sound pressure level of 130–140 dB at a distance of 0.1 m (L. Jakobsen et al., 2013; A. Surlykke & E. K. Kalko, 2008). Because of the proximity of a bat's mouth to its ears, a vocalization immediately reach the ears without being attenuated by the air (B. D. Lawrence & J. A. Simmons, 1982), but will be moderately attenuated by the directionality of the emitters and ears (L. Jakobsen et al., 2013; H.-U. Schnitzler & A. Grinnell, 1977). On the other hand, the intensity of an echo reflected off an insect is decreased due to attenuation in the air (B. D. Lawrence & J. A. Simmons, 1982) and varies with the target strength of the insect. Delay-tuned neurons measure target distance by processing pulse-echo delays within approximately 30 ms (N. B. Kothari et al., 2018). Thus, it is expected that bats' sensitivity to faint echoes is degraded due to masking by proximate intense emissions. A pioneering study by O. Henson Jr (1965) proposed a mechanism to avoid such masking. He recorded cochlear microphonic potentials and stapedius muscle action potentials in echolocating Mexican free-tailed bats; the action potentials of the stapedius muscles indicated that the muscles contracted a few milliseconds before each pulse emission and relaxed within 10 ms, suggesting that the emission energy was reduced in the middle ear. Also, cochlear microphonic potentials were larger in response to echoes than to emitted pulses, suggesting a mechanism to prevent the masking of weak echoes.

Auditory masking, however, can also be caused by the vocalizations of other individuals. For example, the pulses of two bats flying in close proximity could be more intense than their own echoes. Thus, sensitivity to proximate echoes is reduced by forward or backward pulse masking. Therefore, bats need another mechanism to listen to their faint echoes while masking sounds from other bats. In general, when animals emit acoustic signals in the presence of masking noises, they involuntarily increase signal intensity, frequency, and duration. This behavior, termed the Lombard effect, could improve the signal-to-noise ratio of acoustic signals (H. Brumm & D. Todt, 2002; H. Brumm et al., 2004; S. E. Parks et al., 2011). S. R. Hage et al. (2013) found that stationary CF–FM bats increased the sound amplitude and frequency of CF components in response to band-limited noises with various frequency ranges, demonstrating the Lombard effect in echolocating bats. E. Amichai et al. (2015) reported that FM echolocating bats, *Pipistrellus kuhlii*, increase the length and frequency of their pulses in response to recordings of conspecific vocalizations. These studies suggest that the Lombard effect improves the signal-to-noise ratio of echoes in bat echolocation. Pulse duration is also lengthened to employ time integration as a response to masking, effectively increasing the detectability of echoes (P. Heil & H. Neubauer, 2003). However, although the Lombard effect is both simple and effective, it may cause interference from the intense sounds that are emitted in groups of bats.

1. 2. 2. Clutter interference

Bats echolocate to find small prey items, but in natural environments, echoes are reflected not only from prey but also from any detectable object (including the ground, conspecifics, tree leaves, etc.), which creates a stream of echoes (Fig. 1). This stream could disrupt bats' hearing a faint insect echo. Echoes also overlap, such as if an insect echo of 3-ms duration were reflected at a delay of 10 ms from directly in front of a bat, and another echo of the same duration were reflected from a tree at a delay of 10.1 ms from 20 degrees to the bat's right. How do bats extract and process weak insect echoes in the temporally overlapping echo streams? It is reported that bats may utilize the harmonic structures of their emitted pulses to defocus echoes from off-axis objects because of the directionality of different frequencies (M. E. Bates et al., 2011).

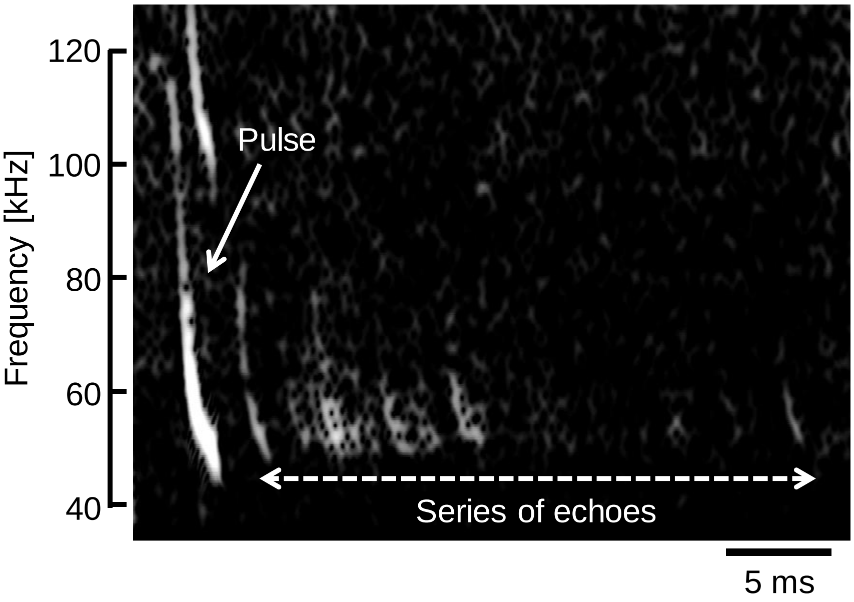


Figure 1-1 Representative spectrogram of an emitted pulse and returning echoes recorded with a telemetry microphone carried by a *Miniopterus fuliginosus* flying in an experimental chamber. Echoes reflected from objects within its echolocation range formed "echo stream".

The stream of echoes poses another perceptual problem: because echolocating bats emit short-interval pulses, successive echoes may continue after the next pulse emission, making it difficult for a bat to distinguish between the echoes of previous and present emissions. This 'pulse-echo ambiguity' could create a virtual image in front of the bat. S. Hiryu et al. (2010) measured echolocation behavior in flying big brown bats using a telemetry microphone while creating the stream of echoes from bunches of hanging chains in an experimental room. In response, the bat shifted the frequency of successive pulses, which were emitted in shorter intervals to avoid pulse-echo ambiguity.

1. 2. 3. Cocktail party nightmare

The time–frequency structure of echolocation pulses is similar within a species, although there are inter-individual differences. When multiple bats are flying in the same vicinity, sounds emitted by all individuals cause not only the problem of auditory masking but also the problem of being confused by other bats sounds. Figure 2A and B show sound spectrograms of echolocation pulses from *Miniopterus fuliginosus* (Fig. 2A) and *Rhinolophus ferrumequinum nippon* (Fig. 2B) flying alone. An echolocating bat emits 10–20 pulses per second, which can create a complex acoustic scene with multiple bats. Figures 2C and D are examples of spectrograms from groups of *M. fuliginosus* (Fig. 2C) and *R. f. nippon* (Fig. 2D). In both cases, conspecific echolocation caused severe acoustic situations where the temporal and spectral overlap of sounds was prominent. This perceptual problem is called a "cocktail party nightmare" (N. Ulanovsky & C. F. Moss, 2008). How bats are able to distinguish their own echoes has fascinated researchers since the discovery of echolocation. The ability of bats to discriminate distances is remarkably degraded in the presence of sounds similar to their own echoes (W. Masters & K. Raver, 1996). It is hypothesized that echolocating bats increase the inter-individual differences among vocalizations to avoid confusing their own sounds with those of conspecifics (jamming avoidance responses; JARs; (W. M. Masters et al., 1991)).

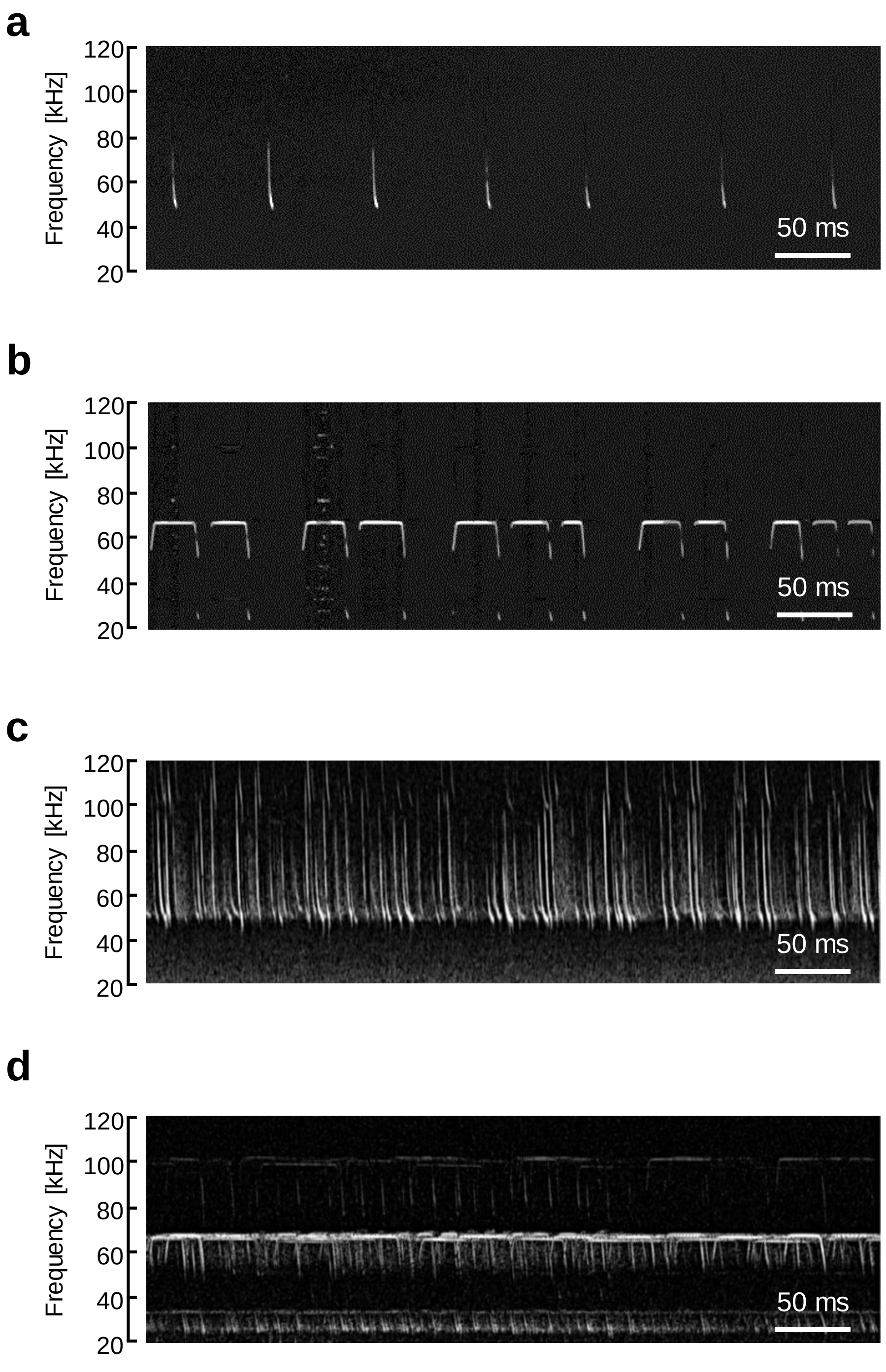


Figure 1-2 “Cocktail party nightmare” of both FM and CF-FM bats. Representative spectrograms of sounds emitted by a single flying *M. fuliginosus* (a) and *R. f. nippon* (b), respectively. When multiple bats flying together, acoustic scene becomes much more severe. In the case of *M. fuliginosus* (c), spectral overlaps of each sound are prominent. In addition to the spectral overlaps, tempral overlaps are also occurred in the case of *R. f. nippon* (d).

J. Habersetzer (1981) demonstrated that *Rhinopoma hardwickei* emitted their pulses in three different frequency bands when flying in conspecific groups but used a single frequency band when flying alone. Furthermore, pairs of *Tadarida teniois* shifted their terminal frequency of their downward FM echolocation pulses when the distance between them was great (symmetric JAR; (N. Ulanovsky et al., 2004)). When a pair is echolocating close together, one bat uses a terminal frequency higher than its baseline, and the other does not shift its terminal frequency (asymmetric JAR). *T. brasiliensis* shift their terminal frequency upward within 200 ms in response to conspecific recordings (E. H. Gillam & B. K. Montero, 2015; E. H. Gillam et al., 2007).

These studies suggested that bats actively change acoustic characteristics of their emitted pulses to facilitate echo segregation from similar sounds generated by conspecifics. However, the data presented in these studies were from measurements taken using fixed microphones. The acoustic characteristics of sound recorded with fixed microphones could be distorted by the Doppler effect or atmospheric attenuation. In addition, the sounds of multiple bats may become distorted by the similarity in pulses among individuals. These methodological problems have constrained our understanding of group echolocation behavior.

In the CF–FM sounds used by *Pteronotus pernellii* for echolocation, the second harmonic is the most prominent in the pulses emitted in the air, but the first harmonic is the strongest in the vocal tract (D. J. Hartley & R. A. Suthers, 1990). Thus, the bat only hears its first harmonic, but the first harmonics of other bats are probably inaudible. A neural gate to process echoes in their auditory system is opened by hearing the first harmonic, reducing confusion in the presence of conspecifics (I. Taniguchi et al., 1986).

Another solution to interference from conspecific vocalizations is to regulate the timing of pulse emission. When exposed to various types of noise bursts, stationary *T. brasilliensis* suppress their emission for over 70 ms after noise presentation (J. Jarvis et al., 2010). However, vocal timing is strongly related to wingbeat (B. Falk et al., 2015), and it is more likely that vocalizations are not made during wingbeats than that the bats flexibly change the timing of pulse emissions. Under acoustic interference, *T. brasilliensis* omitted entire strobe groups (clusters of pulses emitted at short intervals) or decreased the number of pulses per strobe groups (Adams et al., 2017). E. Takahashi et al. (2014) demonstrated that echolocating *Pipistrellus abramus* emitted more pulses in the presence of noise than in silence. It is not yet known whether such variations in pulse emission are related to the wingbeat cycle.

C. Chiu et al. (2008) demonstrated that when two big brown bats echolocated, the trailing individual spent considerably less time vocalizing. This ‘silent behavior’ is useful for avoiding temporal overlaps between pulses and facilitating effective prey capture. The trailing bats showed higher prey capture rates than leading bats when they flew in pair to capture a single prey item (C. Chiu et al., 2010). Echolocating bats can also eavesdrop on others (R. M. Barclay, 1982; D. K. Dechmann et al., 2009).

1.3. Research questions

Although a number of previous studies demonstrated that FM echolocating bats employ JARs in the presence of artificial or conspecific noise, it remains poorly understood. For example, little is known about how acoustic characteristics of interfering sounds such as frequency, duration, or sweep pattern affect JARs; this is important for understanding the key components of recognizing signals in the presence of background noise. Moreover, the use of JARs by bats in groups of three or more is poorly understood. When sounds of multiple bats would create a complex auditory scene, how do they segregate signals? Can they change their frequency to avoid spectral overlap? If changes in spectro-temporal characteristics occur during acoustic jamming, is it likely to reduce the jamming effect?

Echolocation behavior of CF–FM bats in noisy environments has been less studied than that in FM bats. As described above, CF–FM bats exhibit doppler shift compensation. How is echo frequency maintained within a narrow frequency range in the presence of multiple conspecifics? In addition, a comparative investigation is important for a general understanding of how bats extract own echoes from similar sounds generated by other bats.

The masking effect caused by strong emission can be explained by reduced auditory sensitivity due to the contraction of muscles of the middle ear. However, to our knowledge, no study has investigated masking effects caused by sounds of conspecifics. How do bats deal with strong sounds form other bats with unpredictable timing? To answer these questions, I conducted several experiments for my dissertation.

1.4. Dissertation Outline

In the first experiment, echolocation pulses of *M. fuliginosus* were recorded using a telemetry microphone attached to the bat. The acoustic characteristics of echolocation pulses were compared in the absence and the presence of jamming sounds, and between jamming sounds of different frequencies. The results indicated that bats actually changed their pulse characteristics based on the frequency of jamming sounds.

In the second experiment, I investigated acoustic characteristics of *M. fuliginosus* in response to jamming with different frequency-modulated patterns. This experiment showed that echolocating bats shifted their terminal frequency upward in response to an FM sound mimicking bat pulses, a time-reversed version of the FM sound, and CF sounds. They did not change their terminal frequency in the presence of simple, linear upward and downward FM sounds. These results indicate that direct frequency masking induces a TF shift which decreases the similarity between their own echolocation sounds and jamming signals.

In the next two experiments, I measured group echolocating behavior by capturing the vocalizations of each group member. In the third experiment, we compared the behavior of *M. fuliginosus* flying alone with that in a group of four individuals. We found that their terminal frequency shifted when flying in a group. We evaluated the pulses of individuals before and after group flight. We also investigated how similarities between bat-like FM signals were affected when an acoustical characteristic of an FM signal differed from that of the original FM signal. We found that in groups, FM bats shifted their terminal frequencies differentially, an effective way to segregate bat-like signals from other interfering signals.

In the fourth experiment, echolocating *R. f. nippon* were flown singly and in groups of three individuals. We found adaptive changes in acoustic characteristics; they decreased the duration of CF–FM sounds and increased the duration and bandwidth of terminal FM sounds. There was no clear pattern of change in reference frequency (the echo frequency of their pulses maintained within optimal hearing). Shifts in the frequency of pulses emitted by other individuals depended on the relative velocity between individuals flying together, suggesting that their auditory systems, tuned to a narrow frequency range, work as frequency filters to suppress masking by other bats’ sounds.

The final experiment used an electrophysiological technique to quantify the masking effects from conspecific sounds. By recording brainstem auditory evoked potentials (BAEPs) from *P. abramus*, we confirmed forward masking effects on weak FM sounds by preceding stronger FM sounds. The masking effects were reduced by increasing the interval and frequency differences of the two stimuli. Interestingly, the masking effects were reduced by a frequency difference of 0.5 kHz, suggesting that the frequency shifts in response to jamming is useful for reducing the forward masking from sounds of other bats.

Chapter 2: Rapid frequency control of sonar sounds by the FM bat, *Miniopterus fuliginosus*, in response to spectral overlap

In the presence of multiple flying conspecifics, echolocating bats avoid jamming by adjusting the spectral and/or temporal features of their vocalizations. However, little is known about how bats alter their pulse acoustic characteristics to adapt to an acoustically jamming situation during flight. We investigated echolocation behavior in a bat (*Miniopterus fuliginosus*) during free flight under acoustic jamming conditions created by downward FM jamming sounds mimicking bat echolocation sounds. In an experimental chamber, the flying bat was exposed to FM jamming sounds with different terminal frequencies (TFs) from loudspeakers. Echolocation pulses emitted by the flying bat were recorded using a telemetry microphone (Telemike) mounted on the back of the bat. The bats immediately (within 150 ms) shifted the TFs of emitted pulses upward when FM jamming sounds were presented. Moreover, the amount of upward TF shift differed depending on the TF ranges of the jamming sounds presented. When the TF range was lower than or overlapped the bat's mean TF, the bat TF shifted significantly upward (by 1-2 kHz, Student's *t*-test, $P < 0.05$), corresponding to 3-5% of the total bandwidth of their emitted pulses. These findings indicate that bats actively avoid overlap of the narrow frequency band around the TF.

2.1. Introduction

The ability to listen to sounds in noisy environments is important for vocal communication, navigation, and hunting, especially for nocturnal animals that rely mainly on auditory information. Acoustic signal transmissions between transmitters and receivers are affected by ambient noise and are sometimes degraded. Thus, to improve the quality of signal transmission, the acoustic properties of animal emitted sounds are sometimes altered to adapt to the immediate acoustic environment. Primates (H. Brumm et al., 2004; M. Garnier et al., 2010; S. Roian Egnor & M. D. Hauser, 2006; W. V. Summers et al., 1988), birds (H. Brumm & D. Todt, 2002; M. S. Osmanski & R. J. Dooling, 2009; M. Verzijden et al., 2010), and whales (S. E. Parks et al., 2011) are known to change the frequency, syllable duration, and intensity of their vocalization to improve communication efficiency in the presence of background noise.

Bats vocalize ultrasonic pulses and listen to returning echoes to achieve spatial perception in complete darkness. During foraging, echolocating bats change the spectral and/or temporal characteristics of their vocalization to detect information on target prey in weak and cluttered echoes. Many studies have demonstrated such flexibility in bat vocalization under background noise due to the surrounding environment; echolocating bats actively change the acoustic characteristics of their emitted pulses according to the task, in an appropriate way from an acoustical perspective. For example, *Eptesicus fuscus* and *Pipistrellus abramus*, which normally use broadband frequency-modulated (FM) pulses, prolong the terminal frequency portion of the downward FM sweep to concentrate energy of echolocation pulses in the narrow frequency range, resulting in a greater detection range when searching for tiny fluttering insects (S. Hiryu et al., 2008a; E. K. Kalko & H.-U. Schnitzler, 1993; A. Surlykke & C. F. Moss, 2000). When distance to the target prey decreases, FM bat species shorten the pulse duration to avoid temporal overlap with pulses and echoes, but broaden the bandwidth of pulses to improve the temporal resolution of echoes and/or obtain more spectral information about small fluttering insect prey from echoes. Additionally, bats weaken the intensity of emitting pulses as a function of distance to the target prey during normal foraging (E. Fujioka et al., 2011). This decrease in pulse intensity when approaching an intended target (e.g., insect prey or target wall) is referred to as echo intensity compensation, which is thought to stabilize range estimation in the auditory system (S. Hiryu et al., 2007; S. A. Kick & J. A. Simmons, 1984).

The echolocation system of bats should be robust to acoustic interference because the bats have a pressing need to extract as much information as possible from low-amplitude echoes in noisy and cluttered habitats. When bats are foraging with other conspecifics, acoustic jamming results both from pulses and from echoes emitted by other neighboring bats. Nevertheless, even in the presence of other flying conspecifics, bats are able to capture flying insects and avoid surrounding obstacles and neighboring bats. Some previous studies reported that echolocating bats adjust their vocalization to adapt to an acoustically jammed situation caused by other conspecifics. This behavior has been called a jamming avoidance response (JAR). For example, both field and laboratory experiments show that bats change the frequency, duration, and/or timing of emissions to avoid jamming sounds in the presence of conspecifics (C. Chiu et al., 2008; K. Fawcett et al., 2015; K. Fawcett & J. M. Ratcliffe, 2015; J. Jarvis et al., 2013; V. Necknig & A. Zahn, 2011; N. Ulanovsky et al., 2004). Jamming avoidance responses were also demonstrated through elaborate playback experiments in which bats showed changes in the spectro-temporal features of their vocalizations to minimize interference from artificial jamming sounds presented by loudspeakers to stationary bats (M. E. Bates et al., 2008; J. Jarvis et al., 2010). There are also JAR studies of flying bats using such playback techniques (E. H. Gillam et al., 2007; E. Takahashi et al., 2014; J. Tressler & M. S. Smotherman, 2009).

Our present study is one in a series of JAR studies (E. Takahashi et al., 2014), in which bat vocalizations during free flight were monitored by an on-board telemetry microphone mounted on the animal’s back while artificial jamming sounds were presented to control the created acoustically complex situations. E. Takahashi et al. (2014) demonstrated that *Pipistrellus abramus* shifted the TF upward when the stimulus covered the TF ranges of the bat; however, experiments with no overlap between the jamming stimuli and the bat’s TF range were not conducted. To understand how bats respond specifically to overlaps, we increased the variety of stimuli used in the present study. Also, we used a different FM bat species from the previous study with the aim to identify common principles of jamming avoidance behavior by FM bats.

We used *Miniopterus fuliginosus* (eastern bent-winged bat) from the family Vespertilionidae, which is widely distributed throughout southern Asia, including Japan, but its echolocation behavior has not been well investigated. The aim of this study was to test whether *M. fuliginosus* modifies the acoustic characteristics of emitted pulses during flight in response to FM jamming sounds mimicking bat echolocation pulses from loudspeakers. Because our observations showed that *M. fuliginosus* uses FM pulses with a time-frequency structure (see the Results section) very similar to those of *Pipistrellus abramus* (E. Takahashi et al., 2014), we expected that *M. fuliginosus* would also adjust the TF of downward FM pulses for jamming avoidance, as observed in *P. abramus*. If the changes in TF observed in *P. abramus* are also observed in *M. fuliginosus*, this shows that changes in TF are a common adaptation technique in response to acoustic jamming in bats that use FM sounds.

2.2. Materials and Methods

2. 2. 1. Subjects

Seven *Miniopterus fuliginosus* (body mass, 10.4–13.9 g; one male and six females) were used in this experiment. The bats were wild caught from large colonies roosting in natural caves in Hyogo Prefecture, Japan under license and in compliance with current Japanese laws. They were kept in a temperature and humidity-controlled room (4 (L) × 3 (W) × 2 m (H)) at Doshisha University in Kyoto, Japan, and were allowed free access to food (mealworms) and vitamin-enriched water. The day-night cycle of the room was set to 12-h dark/12-h light. *M. fuliginosus* emits downward FM pulses with several harmonics through its mouth. Detailed features of the pulses emitted by *M. fuliginosus* are described in the Results.

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.

2. 2. 2. Experimental procedure

The experiment was performed in an experimental chamber (9.0 m (L) × 4.5 m (W) × 2.4 m (H)) at Doshisha University in Kyoto, Japan. The chamber was constructed of steel plates to minimize interference from external electromagnetic noise and commercial FM radio stations. During experiments, long-wavelength lighting with filters (filtering out wavelengths below 650 nm) was used to prevent the bat from using visual information. The bats flew in a flight space that was delimited by a net suspended from the ceiling and walls (3.0 m (L) × 4.5 m (W) × 2.4 m (H)). Four loudspeakers (Pioneer Corp., PT-R7 III, Kanagawa, Japan, frequency range: 20-80 kHz) were set in each corner of the flight space in the experimental chamber.

The experimental procedure was the same as in a previous study (E. Takahashi et al., 2014), except for the bat species and the variety of jamming sounds. First, an individual bat was flown in the absence of jamming sounds (jamming off 1). Then, the same bat was flown in the presence of jamming sounds simultaneously presented from the four loudspeakers (jamming on) and finally, the bat was flown in the absence of jamming sounds (jamming off 2). During each flight condition, to record the sounds of all bats under equal conditions, sounds were recorded for 6-7 s while the bats performed continuous stereotypical U-turn flights in the chamber. We tested whether flying bats modified the acoustic characteristics of their vocalizations when exposed to jamming sounds.

2. 2. 3. Sound stimuli

The design of the current study was largely similar to our previous study (E. Takahashi et al., 2014), but we used a higher variety of jamming sounds so that we could specifically understand how bats respond to the jamming sounds in the current study. We used computer-generated sounds as jamming sounds. The computer-generated sounds were the same as those used in our previous study on a different FM bat species, *Pipistrellus abramus* (E. Takahashi et al., 2014). This permitted us to compare the results of the present study to those of our previous study. Furthermore, using artificial sounds allowed us to manipulate the TF with a fine resolution. The sounds that mimicked the echolocation pulses emitted by flying FM bats were created using Cool Edit 2000 (Syntrillium Software Corporation, Phoenix, AZ, USA). The jamming sound consisted of a 2-ms FM portion, which was modulated by 40 kHz according to the following equation, after a 1-ms CF portion; thus, the entire duration was 3 ms and the bandwidth was 40 kHz.

$$F(t) = F_{\mathrm{start}} \times \left\{ \left(\frac{F_{\mathrm{end}}}{F_{\mathrm{start}}} \right)^{\frac{1}{\mathrm{duration}}} \right\}^{t}$$

where $F(t)$ is the change in frequency of the FM portion of the jamming sound, F_{start} and F_{end} are the starting and ending frequencies of the jamming sound, respectively, and $\mathrm{duration}$ is the duration of the FM portion. Because bats in flight often change the TFs of emitted pulses, depending on the situation, we created sets of jamming sounds composed of six jamming sounds with different TFs, with TF increasing by 1 kHz between sounds (Fig. 2-1A). The IPI between sounds in each TF series was set at 37 ms, which was the same as our previous study (E. Takahashi et al., 2014). Four sets of jamming sounds were prepared based on the mean TF of *M. fuliginosus*, which was around 47-48 kHz based on our own measurements (see the Results section). The first set consisted of jamming sounds that had lower TFs than the bats' mean TF (TFs of the six sounds ranged from 39 to 44 kHz. See Fig. 2-1B) so that the TFs of the jamming sounds did not overlap with the bat TFs. The TFs of the second set of jamming sounds were lower than the bats' mean TF, with partial overlap (Fig. 2-1C). The third set of TF jamming sounds was higher than the bats' mean TF, with partial overlap (Fig. 2-1D). The fourth set of TF jamming sounds was higher than the bats' mean TF with no overlap (Fig. 2-1E). We examined changes in pulse acoustic characteristics depending on the relationship between bat TF and TF ranges of the sets of jamming sounds. In total, 28 sessions were conducted, using seven bats for each of four sets of jamming sounds.

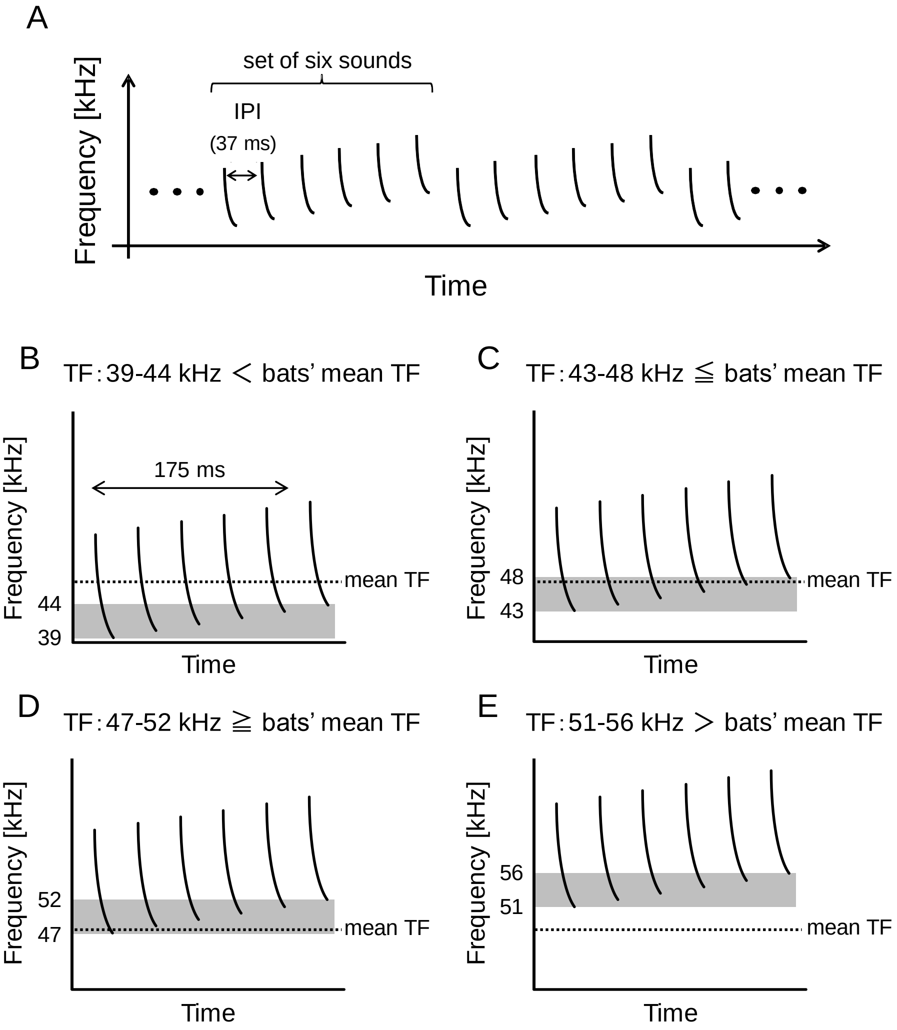


Figure 2-1 Schematic spectrograms of the sequence of jamming sounds used in this study. The jamming sound consisted of a 2-ms FM portion, which was modulated by 40 kHz following a 1-ms CF portion; thus, the entire duration was 3 ms and the bandwidth was 40 kHz. Shadowed areas indicate frequency ranges of the TFs of FM jamming sounds, and dashed lines represent bat's mean TF. (A) Sets of jamming sounds were composed of six jamming sounds with different TFs, increasing by 1 kHz between sounds. The IPI between sounds in each TF series was set at 37 ms, as in our previous study (E. Takahashi et al., 2014). (B) The jamming sounds had lower TFs than the bats' mean TF (TFs of six sounds ranged from 39 to 44 kHz) so that the TFs of the jamming sounds never overlapped with the bat TFs. (C) The TFs of the jamming sounds were lower than the bats' mean TF (TFs of six sounds ranged from 43 to 48 kHz), but partially overlapped. (D) The TFs of jamming sounds were lower than the bats' mean TF (TFs of six sounds ranged from 47 to 52 kHz), but partially overlapped. (E) The TFs of the jamming sounds were higher (TFs of the six sounds ranged from 51 to 56 kHz) and did not overlap the bats' TFs.

Electronic input signals were applied to the four loudspeakers through a high-speed data-acquisition card (National Instruments, Model NI PXle-6358, Tokyo, Japan, 16 bit, fs = 1 MHz) and a 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.

2. 2. 4. Telemike recordings

Echolocation pulses emitted by flying bats were recorded using a custom-made telemetry microphone (Telemike) mounted on the back of the bat. The details of the Telemike recording procedure have been described previously (S. Hiryu et al., 2008b). The Telemike consisted of a ⅛-in omni-directional condenser microphone (Knowles, Model FG-3329, Itasca, IL, 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 Telemike weighed ~0.6 g including the battery. The Telemike was attached to the back of the bat with double-sided adhesive tape, with the microphone pointing forward, between the bat’s ears and 1 cm above the bat’s mouth. The transmitter of the Telemike generated FM radio signals with a carrier frequency between 90 and 105 MHz, which was received by an FM radio antenna (RadioShack Corporation, Model15-1859, TX, USA) that was suspended from the ceiling of the flight chamber. The received signals were demodulated using a custom-made FM receiver, then band-pass filtered between 20 and 150 kHz (NF Corporation, Model 3625, Yokohama, Japan), and digitized using a high-speed data-acquisition card (National Instruments, Model NI PXI-6358, Tokyo, Japan, 16 bit, fs = 384 kHz). The total frequency response of the Telemike system was flat within ±4 dB between 20 and 100 kHz.

2. 2. 5. Sound analysis

The sound analysis was performed as previously described (E. Takahashi et al., 2014). Acoustic characteristics of emitted pulses from flying bats were analyzed from spectrograms from Telemike recordings using custom-written Matlab scripts on a personal computer. In this study, we defined the initial frequency (IF) and TF of each sound as the highest and lowest frequencies in the spectrogram, respectively, that were -

25dB from the maximum energy portion of the spectrogram. The interpulse interval (IPI) and duration were also determined from the spectrogram at -25 dB relative to the maximum energy portion. The BW was calculated by subtracting the TF from IF. The SPL was calculated from the peak to peak amplitude voltage of each pulse in the time domain. We compared SPL between jamming off 1, jamming on, and jamming off 2 during a single flight session for each bat without removing the Telemike so that we could accurately evaluate changes in SPL in response to the jamming sounds.

We tested whether the bat changed the acoustic characteristics of its echolocation pulses in response to jamming sounds using two-way repeated measures ANOVA. If the interaction was significant, a Student’s *t*-test with Holm’s correction was conducted to compare acoustic characteristics between jamming off 1 and jamming on conditions. We also tested whether the acoustic characteristics changed between jamming off 1 and jamming off 2 conditions using two-way repeated measures ANOVA. From seven bats, we compared the mean values of TF, BW, IPI, duration, and SPL of emitted pulses during jamming off 1 and jamming on conditions across four different sets of jamming sounds. *P*-values <0.05 were considered significant. We used SPSS version 23 (IBM, Armonk, New York, USA) for all statistical analyses. Results are presented as mean ± SD.

2.3. Results

2. 3. 1. General echolocation behavior of *Miniopterus fuliginosus*

Miniopterus fuliginosus emitted downward FM pulses with maximum energy at the fundamental component. During free flight in the chamber without jamming sounds, the mean pulse duration and interpulse interval (IPI) were 2.7 ± 0.5 ms and 63.7 ± 10.6 ms, respectively (n = 7 bats), and the mean pulse TF and pulse bandwidth were 47.9 ± 0.6 kHz and 43.3 ± 6.4 kHz, respectively. During the experiments, the bats repeatedly tried to fly in circles in the limited space regardless of the presence or absence of jamming sounds.

The top panel of Figure 2-2 shows spectrograms from a Telemike recording of echolocation pulses emitted by *M. fuliginosus* under jamming conditions constructed by presenting FM sounds with TF ranges between 39−44 kHz. Compared with the input signals of the loudspeakers (bottom panel in Fig. 2-2), the jamming sounds emitted via the four loudspeakers were recorded in sequence by the Telemike, as well as the emitted echolocation pulses and the returning echoes (see solid bars in the top panel). Because the Telemike recorded the sounds the bat actually heard, an acoustic jamming situation could be created at the position of the bat during flight.

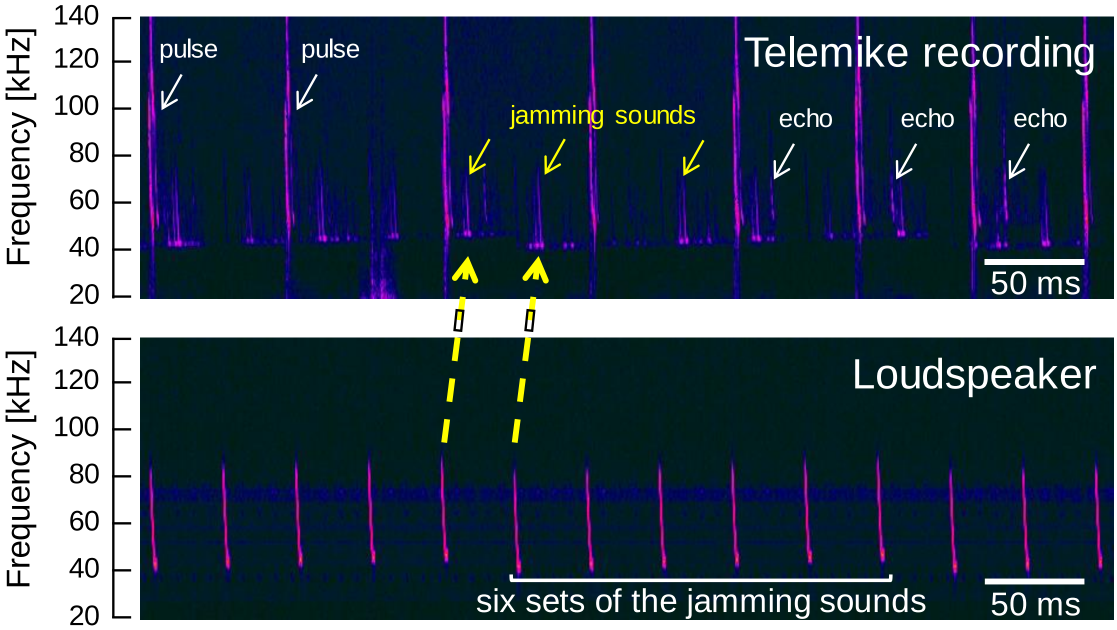


Figure 2-2 Spectrograms from the Telemike recording of echolocation pulses emitted by *M. fuliginosus* during the jamming conditions, constructed by presenting FM sounds with TF ranges of 39-44 kHz (upper) and input electrical signal of the jamming sounds (lower). The jamming sounds emitted via four loudspeakers were recorded in sequence by the Telemike, as were the emitted echolocation pulses and the returning echoes.

2. 3. 2. Changes in acoustic features of echolocation pulses

To determine how fast the bats changed the TF of their emitted pulses, the amount of shift in TF for all bats was normalized as a frequency difference from the means of TFs of each individual measured during the jamming off 1 condition. Figure 2-3 shows the mean shift in TF of emitted pulses in successive 50 ms time bins. The on-response time was defined as the time the mean TF of the bats took to reach 63% of the mean of the TFs during the jamming on condition, and the off-response time was defined as the time the mean TF of the bats took to decrease to 37% of the mean TFs during the jamming on condition. Figure 2A shows that the bats rapidly increased TFs by 1.5 ± 0.4 kHz in response to jamming TFs that partially overlapped the bat TFs (TF range: 43−48 kHz, Fig. 2-1C). Figure 2B shows that the TFs changed by -0.8 ± 0.5 kHz when the loudspeakers were turned off. The on-response occurred within 150 ms, and the off-response occurred within 550 ms. The TF did not return to the mean TF during the jamming off 1 condition within 2 seconds after the loud speakers were turned off.

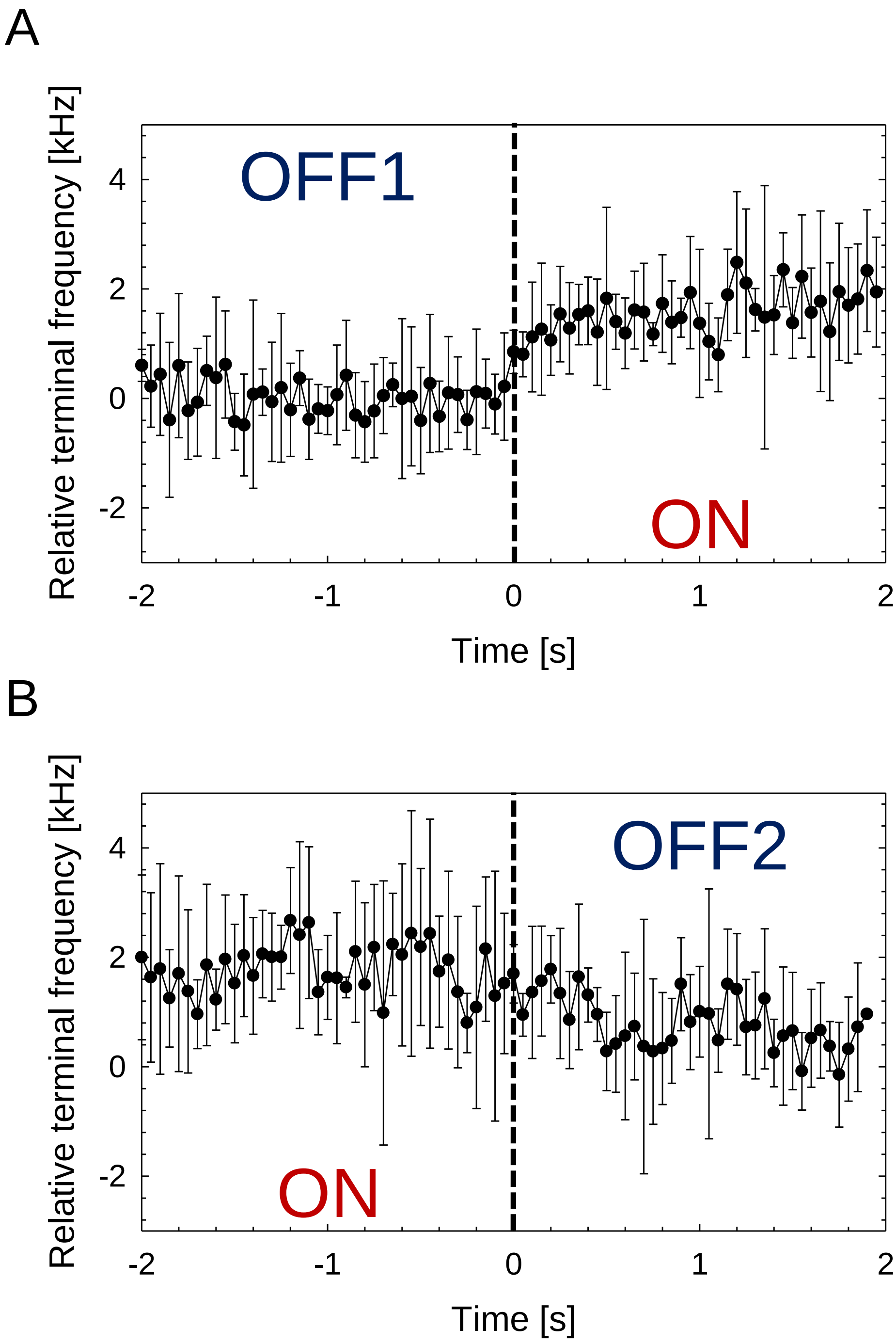


Figure 2-3 (A) The bats rapidly increased TFs by 1.5 ± 0.4 kHz in response to jamming TFs that partially overlapped the bat's TFs (TF range: 43-48 kHz, Fig. 2-1C). (B) TFs changed by -0.8 ± 0.5 kHz when the loudspeakers were turned off. The on-response time occurred within 150 ms, and the off-response time occurred within 550 ms; the TF did not return to the TF mean during the jamming off 1 condition within 2 seconds after the loud speakers were turned off.

Figure 2-4 shows changes in the pulse TF of an individual bat in response to FM jamming sounds with and without jamming (jamming off 1, jamming on, and jamming off 2). The shadowed areas indicate frequency ranges of the TFs of FM jamming sounds presented to the bats. We found that all bats shifted TF upward when exposed to jamming sounds that overlapped and were lower than the mean bat TF (Fig. 2-4A-C). However, bats do not appear to change TF in response to jamming sounds with higher TFs than the mean bat TF (Fig. 2-4D). Figure 2-5 summarizes the results of all individuals shown in Figure 2-4 and the degree to which the bats changed the frequency of their TFs in response to each set of FM jamming sounds compared to no jamming sounds. The amount of upward TF shift differed depending on the TF ranges of the jamming sounds presented (Two-way ANOVA; interaction: $F = 17.998$, $P < 0.05$). *M. fuliginosus* showed a maximum shift (2.0 ± 0.7 kHz) when exposed to jamming sounds whose TFs (43-48 kHz) were lower than, but partially overlapped with, the mean of the pulse TF (range: 47 to 48 kHz for all bats; Student's *t*-test: $t = -7.611$, $P < 0.05$). They also shifted significantly upward by 0.9 ± 0.5 kHz when presented with jamming sounds with TFs of 39-44 kHz (Student's *t*-test: $t = -4.902$, $P < 0.05$), and 1.4 ± 0.7kHz when presented with jamming sounds with TFs of 47-52 kHz (Student's *t*-test: $t = -5.234$, $P < 0.05$). No significant shift (-0.1 ± 0.3 kHz) in TF was observed when the jamming sounds had higher TFs (51-56 kHz; Student's *t*-test: $t = 0.419$, $P = 0.690$).

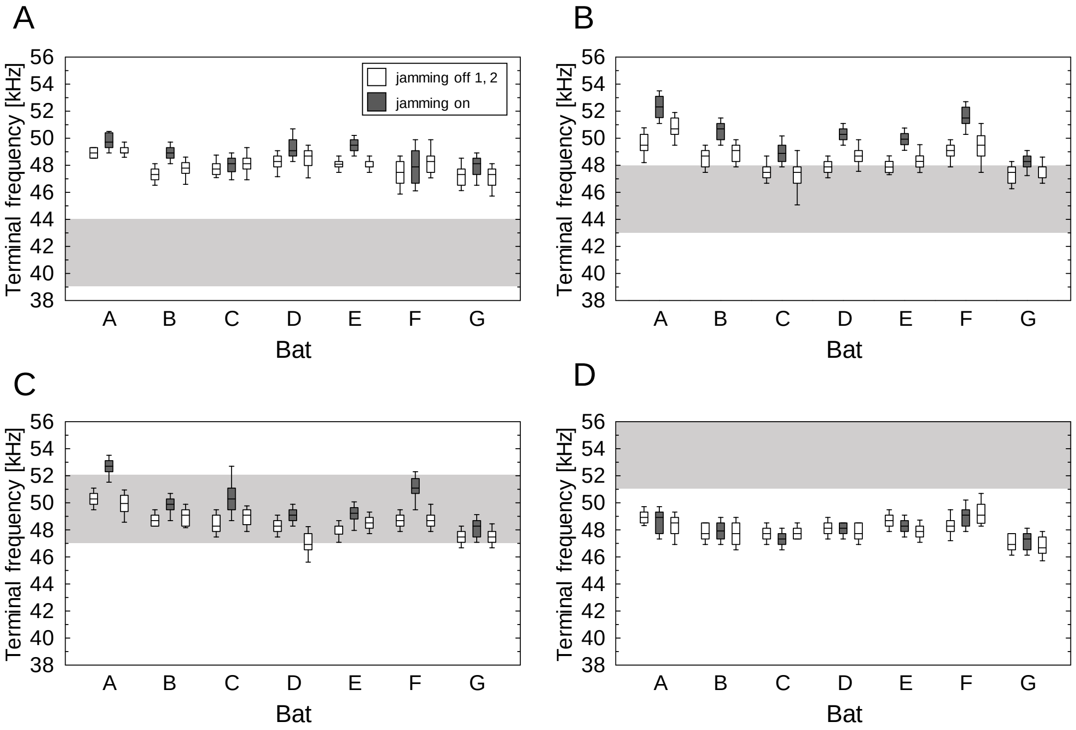


Figure 2-4 Changes in the pulse TF of an individual bat with or without jamming sounds (jamming off 1, jamming on, and jamming off 2). Shadowed areas indicate frequency ranges of the TFs of FM jamming sounds presented to the bats. (A-C) All bat TFs shifted upward when exposed to jamming sounds with TFs lower than and overlapping the bat's mean TF. (D) The bat TF does not appear to change in response to jamming sounds with TFs higher than the mean bat TF.

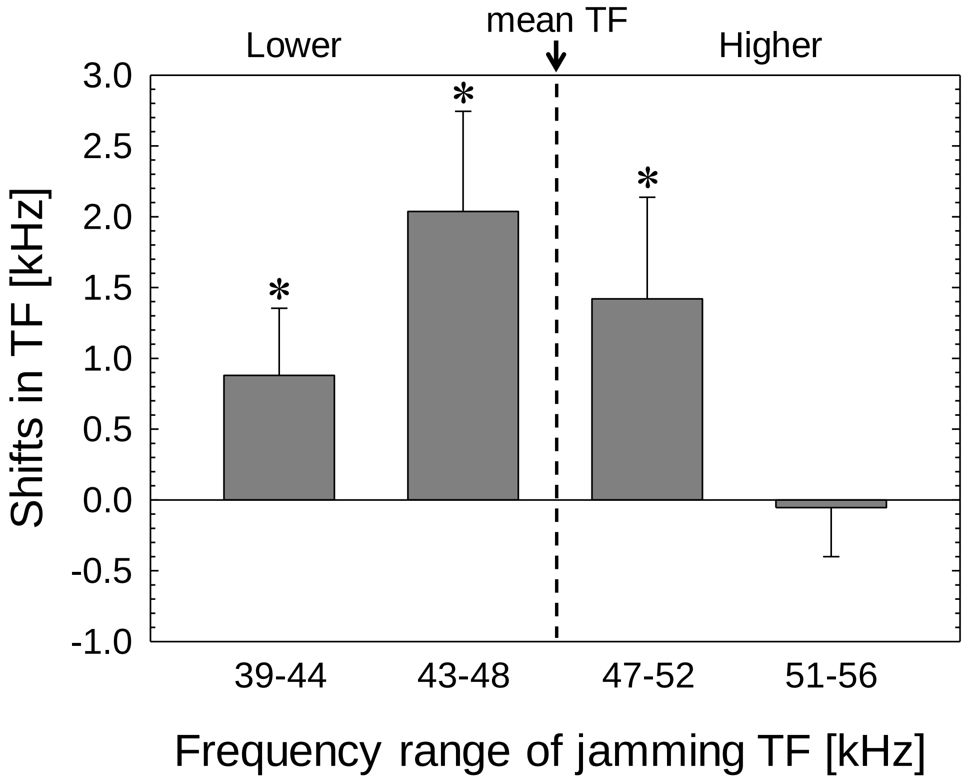


Figure 2-5 Summary of TF shifts with respect to the TF ranges of the presented jamming sounds. The amount of upward TF shift differed depending on the TF range of the jamming sounds (two-way ANOVA; interaction: $F = 17.998$, $P < 0.05$). *M. fuliginosus* showed a maximum shift (2.0 ± 0.7 kHz) when exposed to jamming sounds whose TFs (43□48 kHz) were lower than, but partially overlapped with, the mean of the pulse TF (range: 47 to 48 kHz for all bats; Student's *t*-test: $t = -7.611$, $P < 0.05$). They also shifted significantly upward by 0.9 ± 0.5 kHz when jamming sound TFs were 39□44 kHz (Student's *t*-test: $t = -4.902$, $P < 0.05$) and 1.4 ± 0.7 kHz when jamming sound TFs were 47□52 kHz (Student's *t*-test: $t = -5.234$, $P < 0.05$). No shift (-0.1 ± 0.3 kHz) in TF was observed when the jamming sounds had higher TFs (51□56 kHz; Student's *t*-test: $t = 0.419$, $P = 0.690$).

Table 2-1 shows changes in TF, BW, duration, sound pressure level (SPL), and IPI of the emitting pulses in response to jamming sounds. The changes in BW, duration, IPI, and SPL of the emitted pulses did not significantly depend on the TF ranges of the jamming sounds (two-way ANOVA; interaction: $P > 0.232$). The jamming sounds led to a significant increase in TF, BW, duration, and SPL in bat emitting pulses during the jamming on condition compared with the jamming off 1 condition (two-way ANOVA; main effect of jamming off 1 vs. jamming on: $P < 0.05$). The bats, however, did not significantly change IPI when jamming sounds were presented (two-way ANOVA; main effect of jamming off 1 vs. jamming on, $P = 0.369$). In addition, TF, BW, duration, IPI, and SPL showed no significant differences between jamming off 1 and jamming off 2 conditions (two-way ANOVA; main effect of jamming off 1 vs. jamming off 2: $P > 0.058$). This indicates there was no habituation in the acoustic characteristics of the bat emitted pulses to the jamming sounds.

Table 2-1 Changes in acoustic characteristics (mean ± SD) of sounds emitted by bats with respect to the TF ranges of the presented jamming sounds. Asterisk indicates $P < 0.05$ by ANOVA.

	39-44 kHz		43-48 kHz		47-52 kHz		51-56 kHz		*P* value		
	off	on	off	on	off	on	off	on	TF range	off/on	interaction
TF [kHz]	47.9 ± 0.6	48.8 ± 0.8	48.3 ± 0.8	50.3 ± 1.5	48.6 ± 0.9	50.0 ± 1.5	48.1 ± 0.7	48.1 ± 0.6	<0.05*	<0.05*	<0.05*
BW [kHz]	43.3 ± 6.4	49.1 ± 4.1	46.3 ± 8.9	49.4 ± 11.0	45.5 ± 8.5	49.4 ± 11.6	41.8 ± 7.7	45.2 ± 12.1	0.085	<0.05*	0.585
Duration [ms]	2.7 ± 0.5	2.9 ± 0.6	2.7 ± 0.7	2.9 ± 0.9	2.7 ± 0.8	2.9 ± 0.8	2.5 ± 0.5	2.7 ± 0.7	0.317	<0.05*	0.981
IPI [ms]	63.7 ± 10.6	71.1 ± 18.6	64.3 ± 6.2	69.4 ± 18.2	66.8 ± 11.9	71.6 ± 16.4	65.0 ± 8.8	66.4 ± 13.3	0.810	0.369	0.591
SPL [dB]	-13.6 ± 6.0	-10.8 ± 7.3	-10.1 ± 5.2	-9.1 ± 5.2	-12.1 ± 6.9	-8.3 ± 6.3	-10.7 ± 2.8	-9.4 ± 4.3	0.784	<0.05*	0.232

2.4. Discussion

In this study, we demonstrated that flying bats rapidly change the TF of their emitted pulses depending on the frequency ranges of the TF of the jamming sounds presented. These rapid shifts were considered a "dynamic frequency shift for JAR," which is a change in the TF within seconds while flying with conspecifics in the field (N. Ulanovsky et al., 2004). Thus, rapid shifts in TF to avoid spectral overlap are important for effective bat echolocation, particularly in situations where they are close to conspecifics. The shifts may also help avoid spectral overlap of their echoes with ambient noises and other bioacoustic signals. Furthermore, the on-response in our study occurred within 150 ms, which is consistent with results from previous playback experiments with *Tadarida brasiliensis*, which showed that the bats rapidly shifted the TF upward in the presence of playback stimuli consisting of recorded FM echolocation sounds within 200 ms (E. H. Gillam et al., 2007). On the other hand, we found that the off-response was slower than the on-response (within 150 ms vs. 550 ms), suggesting separate mechanisms for active (on-response) and passive (off-response) adjustments of vocalization frequency during jamming avoidance.

Bats shifted their TF slightly upward in response to jamming sounds with TFs lower than and overlapping the bats' mean TF. In contrast, no shift in TF was observed when the sounds had higher TFs than the bats' mean TF. When searching for insect prey in open spaces, some FM bat species emit long, shallow FM pulses, called quasi-CF pulses, which emphasize the TF portion of pulses (S. Hiryu et al., 2008a; E. K. Kalko & H.-U. Schnitzler, 1993; A. Surlykke & C. F. Moss, 2000). Moreover, the best frequencies (those that evoke neural responses at the minimum threshold by presenting tone bursts in small frequency steps) in the inferior colliculus of FM bats are correlated with the TF ranges that the bat uses for echolocation (M. Ferragamo et al., 1997; K. Goto et al., 2010; S. Haplea et al., 1994). Thus, the narrow frequency band around the TF is very important for FM echolocating bats and bats shift their TFs slightly to avoid frequency overlap with other sounds. *M. fuliginosus* was reported to use quasi-CF pulses when searching for airborne insect prey in open spaces and to use short, deeply modulated FM pulses to precisely locate objects in closed habitats, as other vespertilionid bats do (K. Funakoshi, 2010). Although there is no reported study on neural mechanisms in the auditory system of *M. fuliginosus*, it seems reasonable that the auditory processing of their sonar echoes occurs in a manner similar to other FM bat species. As a slight change in TF was observed in *M. fuliginosus* and *P. abramus*, this suggests that rapid TF adjustments are common adaptations in response to acoustic jamming in bats that use FM sounds. Alternatively, the shifts in TF may have been due to changes in frequency associated with the Lombard effect in response to spectral overlap.

When presented with jamming sounds, *M. fuliginosus* broadened the pulse bandwidth by 4.1 ± 1.2 kHz. Such broadened bandwidths were also reported in other acoustically complex situations caused by broadband noise (J. Tressler & M. S. Smotherman, 2009). These findings indicate that bats increase the amount of spectral information obtained from the returning echo by broadening the bandwidth, because acoustic jamming leads to a lack of information in the echo and more precise information is required in some situations; e.g. collision avoidance with other bats or capturing insect prey during acoustic interference.

Some animals, including humans, monkeys, and birds, are known to extend syllable duration in a noisy environment and this behavior is effective for improving the signal-to-noise ratio of communicative sounds (H. Brumm et al., 2004; M. Garnier et al., 2010; M. L. Leonard & A. G. Horn, 2005; S. Roian Egnor & M. D. Hauser, 2006; W. V. Summers et al., 1988). Moreover, when bats echolocate when they are exposed to noise, they lengthen the duration of their emitted pulses (E. Takahashi et al., 2014; J. Tressler & M. S. Smotherman, 2009). The time and frequency structure of echolocation pulses emitted by *M. fuliginosus* and *P. abramus* are similar (*M. fuliginosus* vs. *P. abramus*; pulse duration: 3 ms vs. 2 ms, modulation of pulse frequency: 40-100 kHz vs. 40-100 kHz). *P. abramus* was reported to lengthen the duration of their emitting pulses to a greater degree, from 1.6 to 2.0 ms (25%) (E. Takahashi et al., 2014), under the same jamming conditions used in the present study. On the other hand, the average pulse duration slightly, but significantly, increased by 0.2 ms (7.4%) in *M. fuliginosus* in response to the same jamming sound conditions. Knowing that these results are statistically significant, we should further investigate whether this difference is biologically significant.

Moreover, there is another way to improve the signal-to-noise ratio of communication signals, namely the Lombard effect: the involuntary regulation of the amplitude of vocalizations in humans and non-human animals under noisy conditions (H. Brumm & D. Todt, 2002; H. Brumm et al., 2004; M. Garnier et al., 2010; M. S. Osmanski & R. J. Dooling, 2009; S. E. Parks et al., 2011; M. Penna & C. Hamilton-West, 2007; S. Roian Egnor & M. D. Hauser, 2006; W. V. Summers et al., 1988). Also, thus far, only a few studies have reported the Lombard effect in echolocating bats (S. R. Hage et al., 2013; J. Tressler & M. S. Smotherman, 2009). In the present study, *M. fuliginosus* increased the sound intensity by 2 dB under jamming conditions, which is the same as the increase in sound intensity by *P. abramus* in response to the same jamming sounds (E. Takahashi et al., 2014). Our results show that bats actively emit more intense and/or longer-duration sounds in the presence of noise. Also, shortening pulse duration may be useful in avoiding temporal overlap with the sounds emitted by other bats in the vicinity. It is possible that bats might shorten pulse duration if we increased the probability of temporal overlap by shortening the IPI of the jamming sounds.

In this study, we investigated the acoustic properties of echolocation pulses in the presence of jamming sounds. The results showed that the FM jamming sounds that mimicked bat echolocation pulses caused rapid shifts in the TF of emitted pulses by flying bats. Moreover, the shifts varied in response to the amount of spectral overlap of the TF. In the future, using computer-generated sounds, we will investigate how bats change their echolocation according to changes in other acoustical parameters (i.e. duration, sweep rate, and similarity) of jamming sounds. Furthermore, we will investigate whether bats respond differently to real bat calls versus computer-generated jamming sounds. There is another possible strategy to avoid or reduce jamming, which is to change the timing of vocalization. Previous studies have reported that animals, such as primates, birds, frogs, and bats, can regulate the timing of their vocalization to minimize acoustic interference (H. Brumm, 2006; T. U. Grafe, 1996; R. Planque & H. Slabbekoorn, 2008; S. Roian Egnor & M. D. Hauser, 2006; S. Roy et al., 2011). When two big brown bats fly together in a laboratory chamber, the bats lengthen their silent time (C. Chiu et al., 2008). Thus, further investigations are required to understand how bats integrate control of both the spectral and temporal features of their vocalization to effectively avoid or minimize acoustic jamming.

Chapter 3: Adaptive frequency shifts of echolocation sounds in *Miniopterus fuliginosus* according to the frequency-modulated pattern of jamming sounds

When flying in a group, echolocating bats have to separate their own echoes from pulses and echoes belonging to other individuals to extract only the information necessary for their own navigation. Previous studies have demonstrated that frequency-modulated (FM) bats change the terminal frequencies (TFs) of downward FM pulses under acoustic interference. However, it is not yet clear which acoustic characteristics of the jamming signals induce the TF shift according to the degree of acoustic interference. In this study, we examined changes in the acoustic characteristics of pulses emitted by *Miniopterus fuliginosus* while presenting jamming stimuli with different FM patterns to the bat flying alone. Bats significantly altered their TFs when responding to downward (dEXP) and upward (uEXP) exponential FM sounds as well as to a constant-frequency (CF) stimulus, by approximately 1–2 kHz (dEXP: 2.1 ± 0.9 kHz; uEXP: 1.7 ± 0.3 kHz; CF: 1.3 ± 0.4 kHz) but not for linear FM sounds. The feature common to the spectra of these three jamming stimuli is a spectrum peak near the TF frequency, demonstrating that the bats shift the TF to avoid masking of jamming sounds on the TF frequency range. These results suggest that direct frequency masking near the TF frequency range induces the TF shift, which simultaneously decreases the similarity between their own echolocation sounds and jamming signals.

3.1. Introduction

The frequency-modulated (FM) pattern of echolocation pulses emitted by bats is generally similar among conspecific individuals. Even in situations involving acoustic interference, the sensing mechanism of the bat appears to work very accurately. For example, even though many bats are flying at the same time, they never collide with each other while capturing small insect prey using echolocation. Therefore, bats likely possess a mechanism for listening to the echoes of pulses emitted by themselves within a complex auditory scene created by the pulses emitted by every conspecific individual flying together.

Because a bat emits a signal intermittently for echolocation, information obtained from echoes is fragmentary. Therefore, bats increase the pulse emission rate to increase the number of acquisitions of target information; yet, this strategy exacerbates any interference. From the instant of pulse emission, bats are thought to have a time window of several tens of milliseconds during which to processes returning echoes (J. A. Simmons et al., 1979). However, separating echo information using only this time window tactic can become difficult in the following cases: 1) during self-jamming conditions when their own echoes from the surroundings overlap temporally with the target echo within the time window, and 2) under conspecific-jamming conditions when a pulse or echo belonging to other conspecific bats overlaps with their own pulse or echo within the time window. For jamming due to a clutter environment, *Eptesicus fuscus* shifts the terminal frequency (TF) of its downward FM sounds by a few kHz, only when echo streams of consecutive emitted pulses (“strobe groups”) temporally overlap and create ambiguity about matching echoes with emission (S. Hiryu et al., 2010). This strategy represents one type of jamming avoidance response (JAR) to assign echoes to their own emissions by shifting the TFs during echolocation. On the other hand, for jamming environments caused by conspecifics, some field experiments have reported that bats avoid interference by changing the frequency of the emitted pulses during foraging flights with conspecifics (J. Habersetzer, 1981; C. Ibáñez et al., 2004; C. F. Moss & A. Surlykke, 2001; V. Necknig & A. Zahn, 2011). In addition to studies of free-flying bats under natural conspecific jamming conditions, playback experiments also found that some FM bats alter their TFs in response to playback of bat-like FM sounds (E. H. Gillam et al., 2007; K. Hase et al., 2016; J. Luo & C. F. Moss, 2017; E. Takahashi et al., 2014), and to artificially generated constant-frequency (CF) or noise stimuli (M. E. Bates et al., 2008; J. Tressler & M. S. Smotherman, 2009). The reported responses to FM sounds were the same as those to CF sounds or noise stimuli (i.e., increasing frequency, duration, or sound pressure level). Recently, E. H. Gillam and B. K. Montero (2015) evaluated the effects of CF and FM sounds followed by CF components of various durations on freely flying *Tadarida brasiliensis* in the field, and reported that the bandwidths of the jamming signals affected the strength of the JAR. However, to our knowledge, no other studies have investigated whether and how FM echolocating bats respond differently to naturalistic stimuli like pulse, tone burst, or noise stimuli.

The majority of previous studies have reported TF shifts under acoustic interference conditions during echolocation. However, it is not yet clear which acoustic characteristics of jamming signals cause the TF shift. In this study, we examined the following two hypotheses: 1) bats shift the TF in response to a jamming signal with an FM pattern very similar to their own echolocation signals, or 2) bats shift their TF any time the TF of the returning echoes is masked acoustically because the TF is important for echolocation in FM bat species. We conducted playback experiments using loudspeakers in a laboratory flight chamber; five jamming stimuli with different FM patterns were presented to free-flying bats so that we could investigate whether the sounds that mimic the entire echolocation pulse have a greater effect than sounds that merely mask the loudest (terminal) portion of the call. Changes in the acoustic characteristics of the pulses under acoustic interference were investigated using a telemetry microphone system (K. Hase et al., 2016). We created four FM sounds as jamming stimuli (downward or upward and exponential or linear) and one CF sound. If the first hypothesis about the cause of the TF shift were supported, bats would be expected to respond only to the downward exponential FM sound, as it most closely matches their own echolocation sound. In contrast, if the second hypothesis were supported, a TF shift would be expected to occur for the downward and upward exponential FM sounds as well as a CF sound at the TF.

3.2. Materials and Methods

3. 2. 1. Subjects

Eight *Miniopterus fuliginosus* (body mass, 10.8–13.4 g; five males and three females) were used in this experiment. The bats were caught in the wild from large colonies roosting in natural caves in Fukui Prefecture, Japan, under license and in compliance with current Japanese laws. Animals were kept in a temperature- and humidity-controlled room (4.0 × 3.0 × 2.35 m; L × W × H) at Doshisha University in Kyoto, Japan. Bats were fed mealworms *ad libitum* and had free access to vitamin-enriched water. The day–night cycle of the room was set to 12-h light and 12-h dark. *Miniopterus fuliginosus* emit downward FM pulses with maximum energy at the fundamental component.

All 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.

3. 2. 2. Experimental procedure

We conducted the experiments in a flight chamber (9.0 × 4.5 × 2.35 m; L × W × H) at Doshisha University in Kyoto, Japan. The chamber was constructed of steel plates to lower the risk of interference from external electromagnetic noise and commercial FM radio stations. During our experiments, long-wavelength lighting with filters (filtering out wavelengths below 650 nm) was used to prevent the bat from using visual information. The bats flew in a flight space that was delimited by a net suspended from the ceiling and walls (3.0 × 4.5 × 2.35 m; L × W × H). We set four loudspeakers (Pioneer Corp., PT-R7 III, Kanagawa, Japan, frequency range: 20–80 kHz) at each corner of the flight space in the experimental chamber. The height of the four loudspeakers was 1 m above the floor, and the direction of each loudspeaker was set to face the center of the flight chamber. The experimental procedure was similar to that used in a previous study (K. Hase et al., 2016). First, an individual bat was flown in the absence of jamming sounds (jamming off). Then, the same bat was flown in the presence of jamming sounds simultaneously presented from the four loudspeakers (jamming on). In this experiment, we recorded about 20–30 s while flying the bat under the jamming off and on conditions (note that the jamming sounds were presented to the bats approximately 10 to 15 s after the bat started to fly). Then, pulses emitted during 10 s under either the jamming-off or -on condition were analyzed. All bats flew in a circular orbit within the chamber. We tested whether flying bats modified the acoustic characteristics of their vocalizations according to the FM patterns of the presented jamming sounds.

3. 2. 3. Sound stimuli

We generated sound stimuli using Matlab 2014a. We created five types of jamming sounds with different FM patterns using the following formula (S. Parsons & G. Jones, 2000):

$$f(t) = \frac{f_0}{f_0 - af_1}\left\{(f_0 - f_1)\left(\frac{af_1}{f_0}\right)^t + (1-a)f_1\right\},$$

where f_0 and f_1 indicate the start and terminal frequencies, respectively, and a is a constant that determines the shape of the sweep. By changing the value of a, jamming sounds with different frequency sweeps (including CF sounds) were created (Fig. 3-1): one CF sound of 45 kHz, which is slightly lower than the average TF (approximately 48 kHz) of this bat species (K. Hase et al., 2018); and four different types of FM sounds with a minimum frequency of 45 kHz, a maximum frequency of 85 kHz, and a bandwidth of 40 kHz: 1) downward exponential (dEXP), 2) upward exponential (uEXP), 3) downward linear (dLIN), and 4) upward linear (uLIN) FM sounds. The signal length of all sounds was 3 ms. In the dEXP jamming sound, the FM pattern falls exponentially in the way of an echolocation FM pulse of *Miniopterus fuliginosus.* The interpulse interval between sounds was set at 50 ms. The sound pressure level of the jamming FM sounds ranged from 110 to 120 dB sound pressure level peak-to-peak at 10 cm from the loudspeaker.

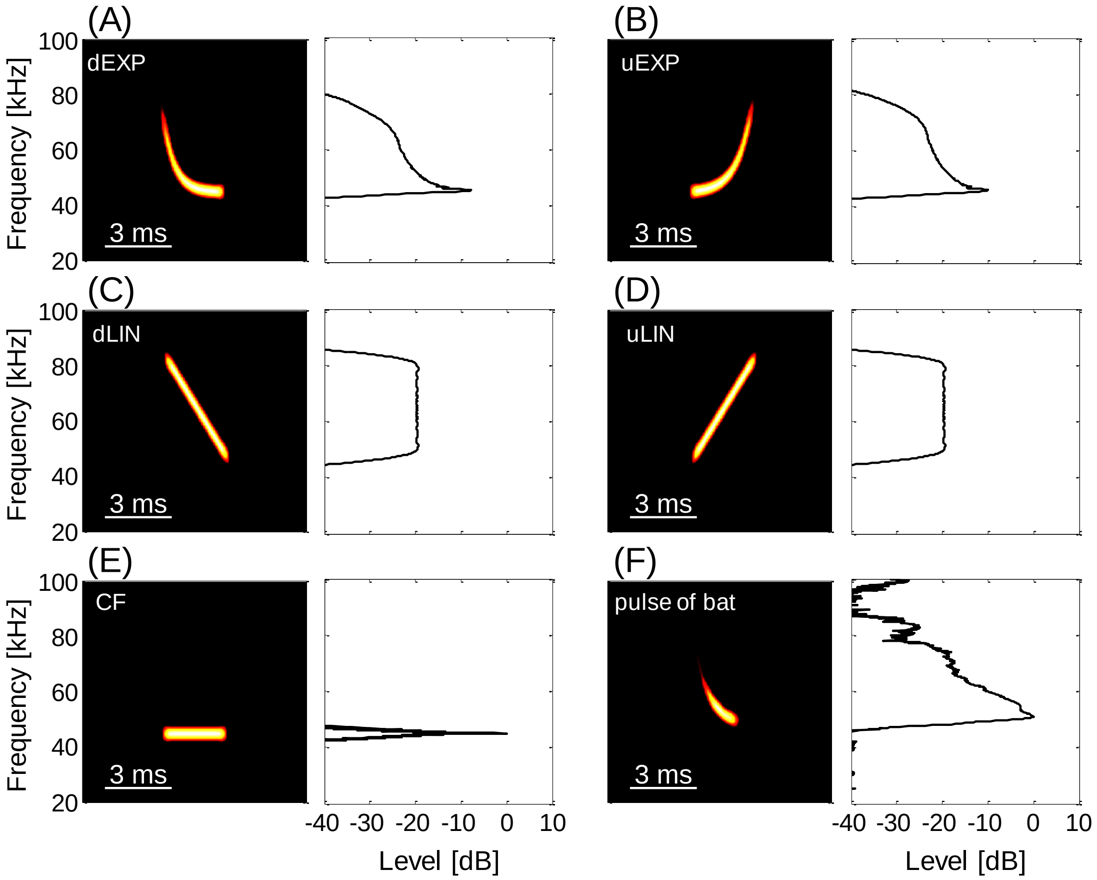


Figure 3-1 Spectrogram (left panel) and spectrum (right panel) of five types of jamming stimuli used in this experiment. (A) Downward exponential (dEXP) jamming sound with a sweep similar to the echolocation pulse of *Miniopterus fuliginosus*. (B) Upward exponential (uEXP). (C) Downward linear (dLIN). (D) Upward linear (uLIN). (E) Constant-frequency (CF) sound at 45 kHz. (F) Example of a typical pulse of *Miniopterus fuliginosus*. The powers in the spectra of five jamming stimuli were normalized to the peak power of CF sounds.

3. 2. 4. Telemike recordings

Echolocation pulses emitted by flying bats were recorded using a custom-made telemetry microphone (Telemike) mounted on the back of the bat (K. Hase et al., 2016). The Telemike consisted of a 1/8-in omni-directional condenser microphone (Knowles, Model FG-3329, Itasca, IL, 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 Telemike weighed ~0.6 g, including the battery. The Telemike was attached to the back of the bat using double-sided adhesive tape, with the microphone pointing forward, between the bat's ears and 1 cm above the bat's mouth. The transmitter of the Telemike generated FM radio signals with a carrier frequency between 76 and 104 MHz, which was received by an FM radio antenna (Terk Technologies Corporation, FM+, Commack, New York, USA) suspended from the ceiling of the flight chamber. The received signals were demodulated using a custom-made FM receiver (ArumoTech Corporation, Kyoto, Japan) and digitized using a high-speed data-acquisition card (National Instruments, Model NI PXI-6358, Tokyo, Japan; 16 bit, fs = 500 kHz). The total frequency response of the Telemike system was flat within ±3 dB between 20 and 100 kHz.

3. 2. 5. Sound analysis

The sound analysis was performed as described previously (K. Hase et al., 2016). The acoustic characteristics of emitted pulses from flying bats were analyzed from spectrograms from the Telemike recordings using custom-written Matlab 2014a scripts on a personal computer. In this study, we defined the initial frequency and TF of each sound as the highest and lowest frequencies in the spectrogram, respectively, that were -25 dB from the maximum energy portion of the spectrogram. The interpulse interval and duration of each sound were also determined from the spectrogram at -25 dB relative to the maximum energy portion. The bandwidth of each sound was calculated by subtracting the TF from the initial frequency. The sound pressure level was calculated from the peak-to-peak amplitude voltage of each pulse in the time domain. We compared the sound pressure level between jamming-off and jamming-on conditions for each bat without removing the Telemike so that we could accurately evaluate changes in sound pressure level in response to the jamming sounds.

We performed Kruskal–Wallis tests to investigate whether a representative bat (Bat A in Fig. 3-3A) changed the TFs of its echolocation pulses in response to jamming sounds. If significant, we then used Mann–Whitney *U* tests with Holm's correction to compare the TFs of pulses emitted under jamming-off and jamming-on conditions in response to the presentation of each stimulus. We also tested whether the mean changes in the acoustic characteristics of all bats under the jamming-on condition differed from 0 in response to each jamming stimulus using a one-sample *t*-test with Holm's correction.

We used SPSS version 25.0 (IBM, Armonk, New York, USA) for all statistical analyses. Results are presented as mean ± SD.

3.3. Results

3. 3. 1. Changes in acoustic characteristics for each stimulus

While exposed to the jamming sounds, the bat was flying in circles in the flight space, and the state of the flight did not change regardless of the presence or absence of jamming sounds, similar to our previous study (K. Hase et al., 2016). The telemetry microphone recorded not only the pulses emitted by bats during flight but also the jamming sounds reaching the bats from the loudspeakers (Fig. 3-2). Figure 3-2 shows that after receiving the dEXP jamming sounds, the bat shifted the TF of the first emitted pulse (see yellow bars).

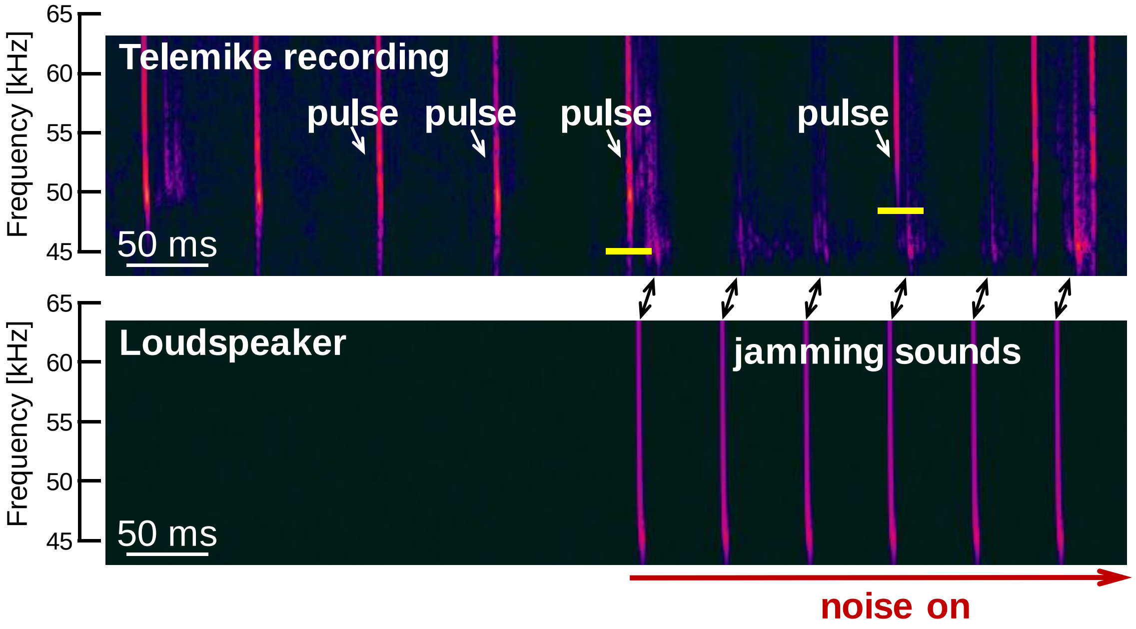


Figure 3-2 Representative sound sequence recorded by the telemetry microphone during flight when dEXP was presented at a 50-ms interval. At the moment of the jamming sound, the bat stopped emitting pulses, then increased the terminal frequency (TF, see yellow bars) of the emitted FM pulse.

Figure 3-3A shows a representative result of one individual. This bat significantly increased the TFs in response to dEXP, uEXP, and CF (mean ± SD, dEXP: from 46.7 ± 0.8 kHz to 47.8 ± 0.9 kHz; uEXP: from 45.9 ± 1.0 kHz to 47.5 ± 0.9 kHz; CF: from 44.8 ± 0.7 kHz to 45.6 ± 1.2 kHz, Mann–Whitney *U* test, $Z < -5.269$, $P < 0.01$). In contrast, the bat did not significantly change the TFs in response to dLIN or uLIN (dLIN: from 46.9 ± 1.0 kHz to 46.9 ± 0.9 kHz; uLIN: from 45.6 ± 1.1 kHz to 45.6 ± 1.1 kHz, Mann–Whitney *U* test, $P > 0.218$). Figure 3-3B shows the changes in TFs for all individuals. We subtracted the mean of the TF of each animal under the jamming-off condition from the TF of each pulse when presenting jamming sounds and then obtained individual means of the TF shift for all pulses of all individuals. Significant changes in TFs were observed for dEXP, uEXP, and CF (dEXP: 2.1 ± 0.9 kHz, n = 8 bats, $t = 6.068$, $P < 0.01$; uEXP: 1.7 ± 0.3 kHz, n = 5, $t = 12.270$, $P < 0.01$; CF: 1.3 ± 0.4 kHz, n = 4, $t = 5.191$, $P < 0.05$), whereas no significant changes in TFs occurred for dLIN and uLIN (dLIN: 0.5 ± 0.7 kHz, n = 7, $t = 1.732$, $P = 0.197$; uLIN: 0.5 ± 0.4 kHz, n = 5, $t = 2.145$, $P > 0.134$).

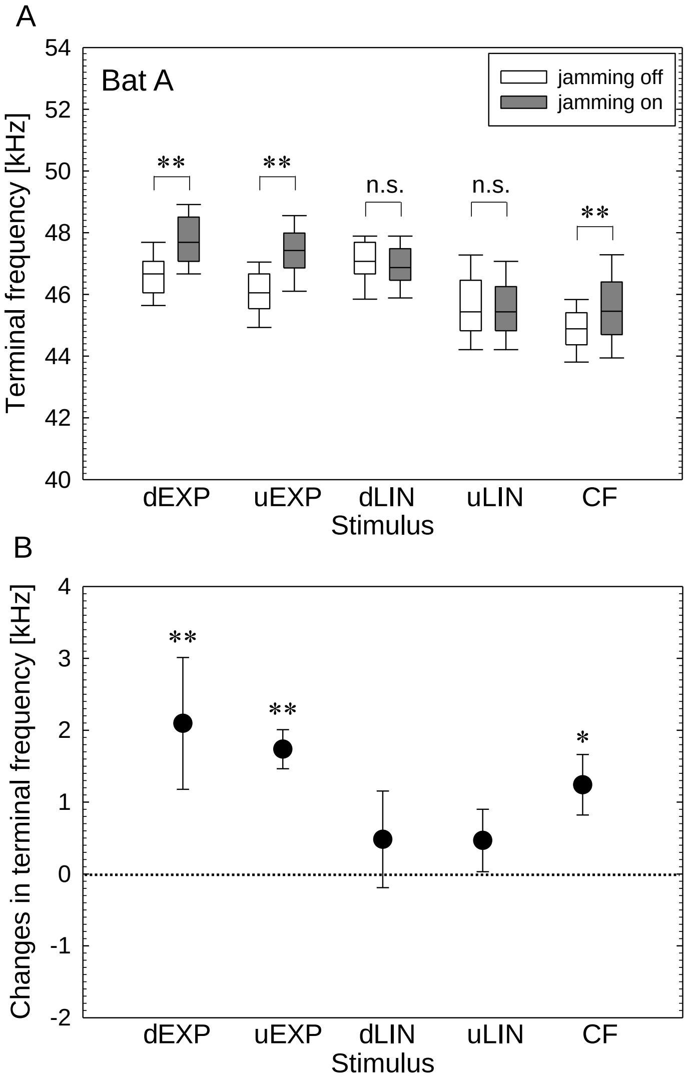


Figure 3-3 Comparisons of changes in TFs among five patterns of jamming stimuli in Bat A (A). Each boxplot shows the results of pulses emitted during 10 s under either the jamming-off or -on condition. Bat A significantly changed its TFs in response to dEXP, uEXP, and CF (Mann–Whitney *U* test with Holm's correction, $P < 0.01$), whereas linear FM sounds (dLIN and uLIN) did not induce a TF shift (Mann–Whitney *U* test with Holm's correction, $P > 0.218$). (B) Comparisons of changes in TFs among five patterns of jamming stimuli for all bats. Significant changes in TFs were observed for dEXP, uEXP, and CF (dEXP: 2.1 ± 0.9 kHz, n = 8 bats; uEXP: 1.7 ± 0.3 kHz, n = 5; CF: 1.3 ± 0.4 kHz, n = 4, one-sample *t* test with Holm's correction, $P < 0.05$), whereas no significant changes in TFs occurred for dLIN and uLIN (LIN: 0.5 ± 0.7 kHz, n = 7; uLIN: 0.5 ± 0.4 kHz, n = 5,one-sample *t* test with Holm's correction, $P > 0.134$). Results were presented as Mean ± SD. (The number of pulses analyzed in this study: dEXP;1889 pulses, uEXP; 1512 pulses, dLIN; 1662 pulses, uLIN; 1532 pulses, CF; 1027 pulses).

Figure 3-4 presents changes in the sound pressure level, duration, bandwidth, and interpulse interval of all individuals. Regardless of the pattern of jamming sounds, the change in sound pressure level was not significant (dEXP: 1.2 ± 1.7 dB; uEXP: -0.8 ± 1.4 dB; dLIN: 0.5 ± 2.3 dB; uLIN: 0 ± 0.9 dB; CF: -0.6 ± 0.8 dB; one-sample *t* test, *P* > 0.696, Fig. 3-4A). Similarly, no significant changes were observed for the duration of the emitted pulse (dEXP: 0.1± 0.2 ms; uEXP: 0 ± 0.3 ms; dLIN: 0 ± 0.2 ms; uLIN: 0.2 ± 0.1 ms; CF: 0 ± 0.3 ms; one-sample *t* test, *P* > 0.262, Fig. 3-4B). Although bandwidth slightly expanded in response to the jamming sounds, none of the changes was significant (dEXP: 2.8 ± 2.7 kHz; uEXP: 1.9 ± 3.7 kHz; dLIN: 1.9 ± 6.5 kHz; uLIN: 1.5 ± 3.2 kHz; CF: 0.3 ± 3.1 kHz, one-sample *t* test, *P* > 0.220, Fig. 3-4C). Similarly, no significant changes in the interpulse interval were observed in response to the different jamming sounds (dEXP: 4.4 ± 6.6 ms; uEXP: -5.1 ± 5.0 ms; dLIN: 1.9 ± 7.3 ms; uLIN: 7.1 ± 6.8 ms; CF: 10.9 ± 4.8 ms; one-sample *t* test, *P* > 0.151, Fig. 3-4D).

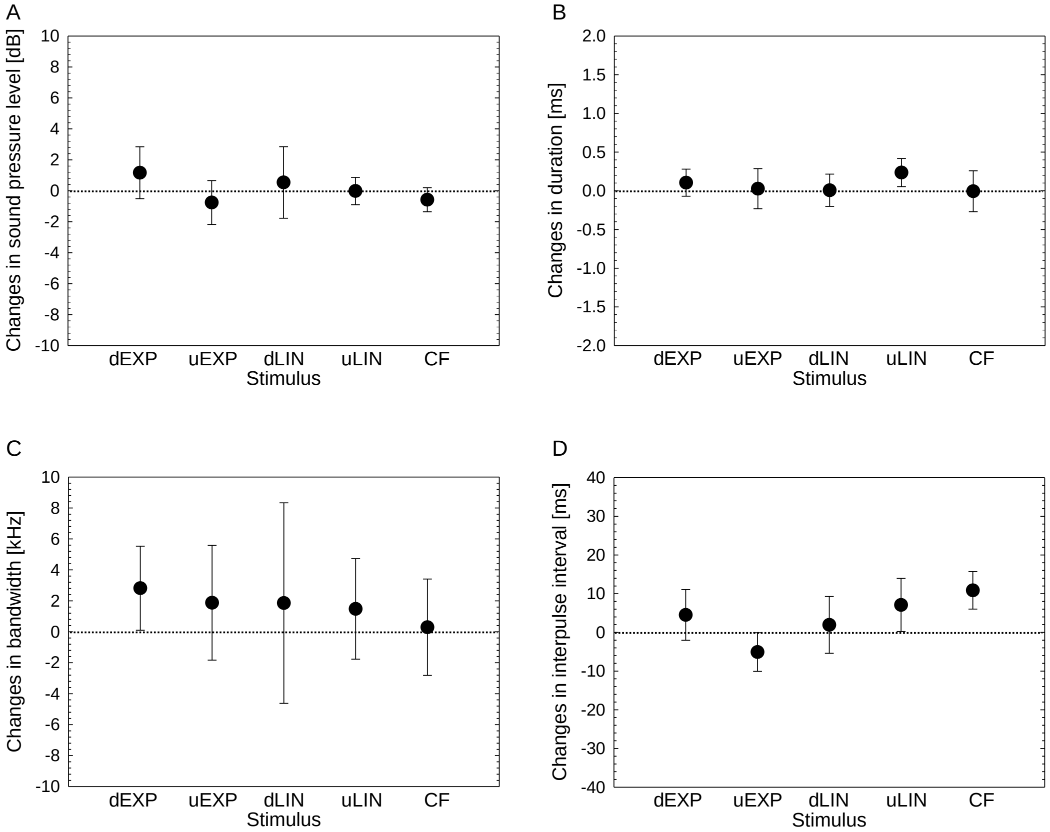


Figure 3-4 Comparisons of changes in the acoustical parameters of an emitted pulse among five patterns of jamming stimuli for all bats. (A) Sound pressure level. (B) Duration. (C) Bandwidth. (D) Interpulse interval. No significant changes were observed for sound pressure level (one-sample *t* test with Holm's correction, $P > 0.696$), duration (one-sample *t* test with Holm's correction, $P > 0.262$), bandwidth (one-sample *t* test with Holm's correction, $P > 0.220$), or interpulse interval (one-sample *t* test with Holm's correction, $P > 0.151$) in response to any of the stimuli.

3. 3. 2. Rapid TF shift of bats responding to jamming stimuli

Figures 3-5A and B show the mean shifts in TFs of emitted pulses of all bats in successive 50-ms time bins when presenting dEXP and dLIN. For dEXP, an obvious shift of the mean of the TF occurred, whereas no such TF shift occurred in the presence of dLIN. To determine how rapidly the bats changed the TF in response to the jamming sounds, the amount of the shift in TF for all bats was normalized as a frequency difference from the means of TFs of each individual measured during the jamming-off condition. For the responses to jamming sounds other than dLIN and uLIN (which did not cause obvious TF shifts), we calculated the on-response time, which was defined as the time the mean TF of the bats took to reach 63% of the mean of the TFs during the jamming-on condition (K. Hase et al., 2016). By fitting a curve to the data for the temporal change in TFs for all individuals (e.g., Fig. 3-5), the on-response time was 140 ms for dEXP, 50 ms for μEXP, and 320 ms for CF, respectively.

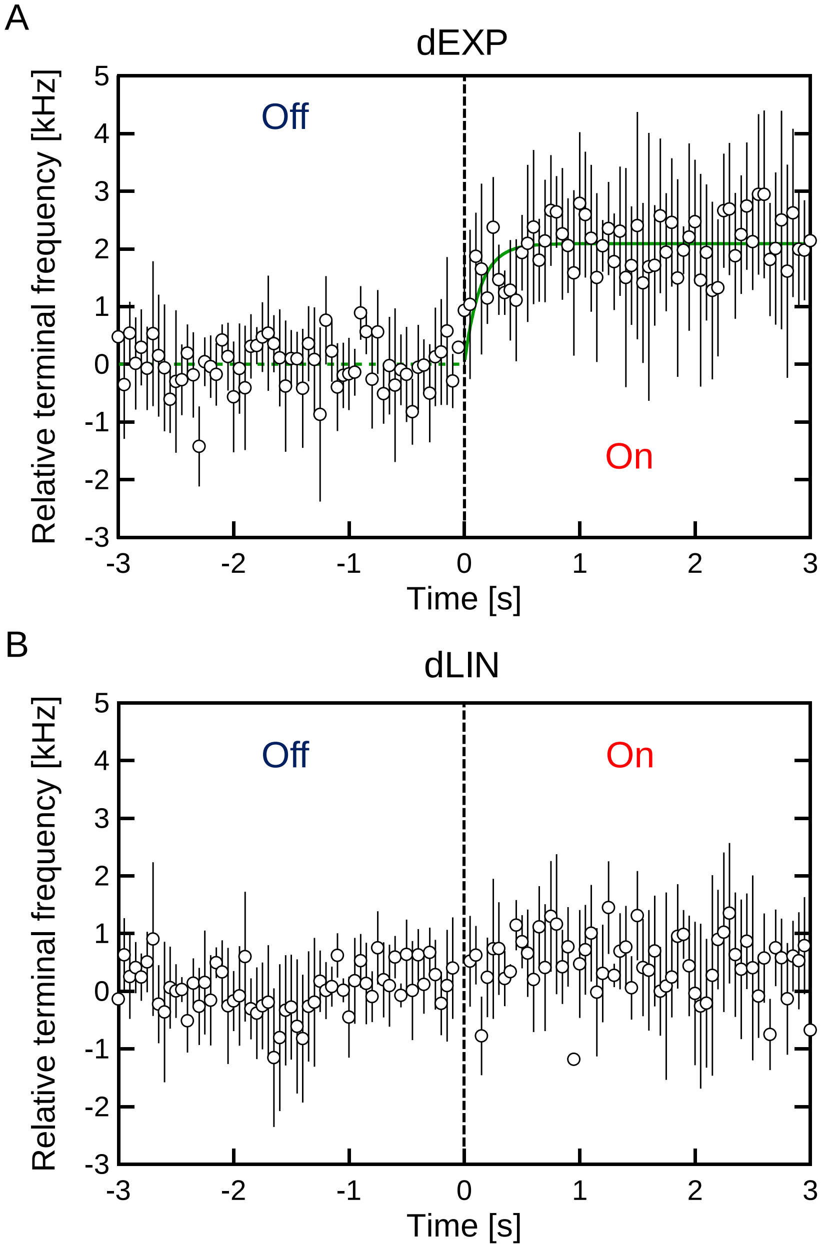


Figure 3-5 Temporal changes in the TFs for dEXP (A) and dLIN (B). The plot shows the mean value of the TFs for all individuals in each 50-ms time bin. The green solid line in (A) is the curve fitted to the data.

3.4. Discussion

3. 4. 1. Acoustic characteristics of jamming signals that impact the TF shift

Previous studies have reported TF shifts by FM-echolocating bats under acoustic interference; e.g., when flying with other conspecifics in the field (J. Habersetzer, 1981; C. Ibáñez et al., 2004; C. F. Moss & A. Surlykke, 2001; V. Necknig & A. Zahn, 2011) or under artificial clutter conditions created by playback experiments using jamming sounds (M. E. Bates et al., 2008; E. H. Gillam & B. K. Montero, 2015; E. H. Gillam et al., 2007; K. Hase et al., 2016; J. Luo & C. F. Moss, 2017; E. Takahashi et al., 2014). However, few studies have addressed how the pattern of the FM of jamming signals impact the degree of the TF shift (E. H. Gillam & B. K. Montero, 2015; T. K. Jones et al., 2018). Therefore, in the present study, we created five jamming sounds with different FM patterns to compare the behavioral response (i.e., the TF shift, as an index to determine which types of acoustic features of jamming sounds directly induce the TF shift in *Miniopterus fuliginosus* during free flight). For all bats used in the experiment, our results indicated significant TF shifts in response to only dEXP, uEXP, and CF and not to dLIN and uLIN. Because the bats exhibited a TF shift in response to the uEXP jamming sound, our findings did not support our first hypothesis, which predicted that bats shift the TF because the jamming signal is similar to the FM pattern of their own echolocation signals.

Figure 3-1 shows that the spectral pattern of uEXP is the same as that of dEXP, which mimics the FM-echolocating sounds of *Miniopterus fuliginosus*. Although the response was slightly weaker compared to dEXP and uEXP, a significant change in the TF was also observed in response to CF jamming sounds. The common feature among the spectra of these three jamming stimuli is a spectrum peak near the TF. Our results may support the second hypothesis that bats shift their TF to avoid the masking of jamming sounds when the TF of the returning echoes is masked acoustically.

3. 4. 2. Response time of the TF shift

In the present study, the on-response time was 140 ms for dEXP and 50 ms for uEXP, respectively. A recent study documented that *Eptesicus fuscus* rapidly shifted the TF of the first vocalization in response to jamming stimuli (FM sounds with three harmonics mimicking echolocation calls of *Eptesicus fuscus*) in the range of 66 to 94 ms (J. Luo & C. F. Moss, 2017). Bats also reportedly change their frequency within 200 ms after FM-jamming sounds are presented in the field (E. H. Gillam et al., 2007). E. H. Gillam and B. K. Montero (2015) also demonstrated that the bandwidth of the jamming signal impacted the response of the observed JAR, and the CF playback did not induce a significant TF shift. In the present study, the response to CF jamming sounds was significant but slightly weaker than the responses to dEXP and uEXP (the on-response time of the CF jamming sounds was estimated to be 320 ms). These findings suggest that the type of jamming stimulus affects the response time and the degree of the TF shift.

Improving the signal-to-noise ratio by raising their own sound pressure level is a result of what is called the Lombard effect. The response latency of the Lombard effect has been estimated to be about 150–175 ms for humans (J. J. Bauer et al., 2006; T. H. Heinks-Maldonado & J. F. Houde, 2005) and 150 ms for birds (M. S. Osmanski & R. J. Dooling, 2009). Bats reportedly increase both the amplitude and frequency of the first call emitted after jamming sounds are presented, indicating that the Lombard effect occurs on a rapid time scale (S. R. Hage et al., 2013; J. Luo et al., 2017). The range of the response latency due to the Lombard effect was similar to the observed on-reaction time in the present study. However, no significant changes in the sound pressure level or duration were observed in the presence of jamming sounds in the present study (Fig. 3-4), suggesting that the observed TF shift was not a reaction to the Lombard effect. Furthermore, a previous study documented that bats were able to exhibit independent shifts between frequency and amplitude under ambient noise (S. R. Hage et al., 2013). Based on these observations, we suggest that bats can avoid spectral masking of the near-TF frequency range by shifting the TF without directly improving the signal-to-noise ratio of the echoes by raising the sound pressure level or lengthening the duration of emitted pulses.

3. 4. 3. Solutions to acoustic interference in FM-echolocating bats

E. Amichai et al. (2015) conducted an experiment in which various jamming sounds from loudspeakers were presented to *Pipistrellus kuhilii* during a landing flight task. Even against a time-reversed echolocation sound where the spectral content was unaffected, calling louder and longer was perceived to be identical to other jamming sounds, which appeared to increase the signal-to-noise ratio of echoes received for echolocation. On the other hand, in *Eptesicus fuscus*, no changes in the target detection capability were observed for a time-reversed playback echo, but the capacity of range discrimination decreased compared to a normal echo (W. M. Masters & S. C. Jacobs, 1989). These previous findings confirm that similarities in the FM pattern between sounds, and not the simple spectral pattern, cause jamming of echolocation at a higher order, such as target ranging, which utilizes the template of their own echolocation sounds (W. Masters & K. Raver, 1996; W. M. Masters & K. Raver, 2000). In fact, during group flight, *Miniopterus fuliginosus* reportedly extend the frequency difference in individual TFs while increasing the intensity of emitted pulses and lengthening the pulse duration (K. Hase et al., 2018). Furthermore, K. Hase et al. (2018) also demonstrated that the slight TF shift decreases the similarity between the dEXP signals. Taken together, echolocating bats could employ various strategies to avoid acoustic interference; they could improve the signal-to-noise ratio of their echoes by emitting louder and longer pulses, which is referred to as the Lombard effect. In addition, they presumably shift the TFs to avoid spectral masking that simultaneously results in decreases of the similarity between signals, which is necessary for “higher-order” echolocation, which may require comparison of the returning echo to a neural template of their own emitted echolocation sounds at higher-order stages of auditory processing. Our findings will help researchers to understand how bats recognize and interpret their own echoes by adaptively changing the acoustic parameters of echolocation sounds.

3.5. Conclusion

We presented five types of jamming sounds with different FM patterns for *Miniopterus fuliginosus* flying alone. For the stimuli that were similar in spectrum to the pulse emitted by the bats and for the stimulus with a constant frequency, *Miniopterus fuliginosus* shifted their TFs. However, no changes were observed for linear FM stimuli. We also measured several other parameters, such as sound pressure level, duration, bandwidth, and interpulse interval, but the bats did not significantly change these parameters in response to the experimental stimuli. Our findings indicate that direct frequency masking near the TF frequency range induced the TF shift in *Miniopterus fuliginosus*, which simultaneously results in the reduced similarity between the signals produced by their own echolocation sounds and the jamming sounds.

Chapter 4: Bats enhance their call identities to solve the cocktail party problem

Echolocating bats need to solve the problem of signal jamming by conspecifics when they are in a group. However, while several mechanisms have been suggested, it remains unclear how bats avoid confusion between their own echoes and interfering sounds in a complex acoustic environment. Here, we fixed on-board microphones onto individual frequency-modulating bats flying in groups; we found that group members broaden the inter-individual differences in the terminal frequencies of pulses, thereby decreasing the similarity of pulses among individuals. To understand what features most affect similarity between pulses, we calculated the similarity of signals mimicking pulses; we found that the similarity between those artificial signals was decreased most by manipulation of terminal frequency. These results demonstrate that the signal jamming problem is solved by this simple strategy, which may be universally used by animals that use active sensing, such as echolocating bats and electric fish, transcending species and sensory modalities.

4.1. Introduction

Animals use acoustic signals to communicate with conspecifics (K. I. Kobayasi et al., 2012), attract females (K. Hammerschmidt et al., 2009; M. J. Ryan et al., 1981), or detect food by hearing prey-generated sounds (R. S. Payne, 1971; H.-U. Schnitzler & E. K. Kalko, 2001). When acoustic communication occurs among a large group of individuals, multiple sound sources produced by conspecifics create a complex auditory scene, presenting what is known as the cocktail party problem (A. W. Bronkhorst, 2000; V. Nityananda & M. A. Bee, 2011; A. K. Schmidt & H. Römer, 2011; N. Ulanovsky & C. F. Moss, 2008). A similar situation can occur when multiple individuals of a species using active sensing emit signals to scan the surrounding environment. For example, in the presence of conspecifics, weakly electric fish create differences in the frequencies of their self-generated electric fields to solve the signal jamming problem; this is called the jamming avoidance response (JAR) (T. H. Bullock et al., 1975). However, although several mechanisms have been suggested, it is still incompletely understood how the cocktail party problem is solved by other animal species that use active sensing, such as echolocating bats. Bats use the echoes of self-generated acoustic signals to hunt, navigate, and orient themselves in total darkness. Bats also hunt or navigate with a number of conspecifics (N. Cvikel et al., 2015a; D. K. Dechmann et al., 2009; E. H. Gillam et al., 2010). Because echolocating bats actively emit signals to scan their environments, groups of bats flying together experience acoustical interference caused by echoes from irrelevant directions and signals belonging to conspecifics (S. Hiryu et al., 2010; C. F. Moss & A. Surlykke, 2001). Under such circumstances, they need to extract biologically relevant sounds from noise and process them to avoid obstacles or to capture food. Understanding the acoustic behavior of group-flying bats would help to reveal how animals acquire acoustic information of interest in a complex auditory environment.

Within the same bat species, the acoustic characteristics (e.g., intensity, bandwidth, and duration) and emission timing of echolocation pulses are generally similar. For instance, identification at the species level is based on the acoustic characteristics of echolocation pulses (S. Parsons & G. Jones, 2000). Therefore, when multiple other individuals are flying in the vicinity, echolocating bats must extract their own echoes from others that have similar characteristics in both the time and frequency domains.

However, echolocation pulses emitted by bats exhibit a certain degree of difference among individuals (E. Amichai et al., 2015; W. M. Masters et al., 1995). Bats can use these differences to discriminate the echolocation pulses of individual bats (Y. Yovel et al., 2009). It has been speculated that echolocating bats broaden inter-individual differences, e.g., the terminal frequencies, duration, and/or sweep rate of emitted sounds, to avoid confusing their own sounds with those of conspecifics (W. M. Masters et al., 1991). However, while most previous studies have focused on changes in the acoustic features of pulses under acoustic interference, there have been no studies in which the inter-individual differences between pairs of individuals were directly measured, i.e., by utilizing on-board microphones, which can separately measure the pulses of bats flying in groups.

JARs have been reported in many species of bats. For instance, previous studies demonstrated that *Tadarida* bats flying in the field shifted their terminal frequencies in response to other bats or to echolocation pulses of the same species presented through a speaker (E. H. Gillam et al., 2007). These findings indicate the possibility that echolocating bats change the acoustic characteristics of their emitted pulses in the presence of pulses from other bats. To the best of our knowledge, however, no previous report has directly measured pulses emitted by each bat flying in a group of more than two individuals. The conventional recording methodology with fixed microphones can be used to identify individuals, especially over short distances, but one must take into account that the recorded sounds will be distorted to some extent (e.g., by the Doppler effect and atmospheric attenuation). Recently, we have used miniature on-board microphones, which measure the sounds of bats without distortion, to investigate echolocation pulses emitted by flying bats under acoustic jamming conditions; we have shown that frequency-modulating bats shift their terminal frequencies during flight depending on the frequency of presented pulse mimics (K. Hase et al., 2016; E. Takahashi et al., 2014). This technique can directly capture how each bat flying *in a group* changes its pulse characteristics to avoid jamming, and the present study is the first to demonstrate experimentally the relationship between the terminal frequencies of pairs of individuals during group flight. We found that the bats broadened inter-individual differences in terminal frequency during group flight and that the similarity of pulses between individuals in a group decreased in group flight. Our computation also revealed that the similarity between bat-like frequency-modulated signals decreased the most with manipulation of terminal frequency. The results suggested that echolocating bats flying in groups broaden the inter-individual differences in terminal frequency in order to decrease the similarity of pulses between individuals. This frequency-shifting jamming avoidance response may be universally used by animals using active sensing, such as echolocating bats and electric fish, transcending species and sensory modality borders.

4.2. Results

4. 2. 1. Groups of bats broaden differences in terminal frequency

To understand how group-flying bats adapt their echolocation behavior in response to acoustic jamming by the pulses of conspecifics, we measured the echolocation behavior of *Miniopterus fuliginosus* flying individually and in groups of four bats. We created six groups of four bats by randomly assigning 19 bats to groups, with some overlaps (Table 4-1). The bats were subjected to three experimental conditions: single flight 1, group flight, and single flight 2. We used miniature on-board microphones (Telemikes) to capture the echolocation pulses emitted by each bat. Video recordings were made in order to reconstruct the three-dimensional coordinates of each bat during flight. We successfully recorded the echolocation pulses and flight trajectories of individual bats when all four bats in a group were flying together in the same flight space (Fig. 4-1). The bats continued flying (without landing) because they were not trained to perform any particular behavioral task. During group flights, no bat ever collided with another individual (Fig. 4-1a). Figure 4-1b shows spectrograms of the echolocation pulses emitted by each individual bat during the 250-ms time intervals indicated by the colored lines on the trajectories shown in Figure 4-1a. The pulses of other bats flying nearby were occasionally recorded by the Telemikes (white triangles in Fig. 4-1b). We examined how the bats changed their pulse characteristics in group flights in comparison with single flights.

Table 4-1 The IDs of the four bats in each group and their terminal frequencies (means ± SDs) during single flight 1.

Group	Bat ID and terminal frequency [kHz]			
1	DBT0200 45.4 ± 0.8	DBT0211 46.1 ± 0.9	DBT0201 47.1 ± 0.5	DBT0206 47.6 ± 1.1
2	DBT0201 46.4 ± 0.8	DBT0208 47.7 ± 1.0	DBT0206 47.9 ± 0.7	DBT0202 49.0 ± 1.1
3	DBT0222 46.1 ± 0.6	DBT0225 46.7 ± 0.4	DBT0226 47.3 ± 0.5	DBT0229 47.4 ± 0.7
4	DBT0217 46.3 ± 1.4	DBT0220 48.5 ± 0.6	DBT0226 48.5 ± 0.8	DBT0224 48.9 ± 0.7
5	DBT0223 46.3 ± 0.4	DBT0222 46.4 ± 0.5	DBT0217 46.4 ± 0.6	DBT0228 46.7 ± 0.7
6	DBT0247 46.1 ± 0.6	DBT0248 46.9 ± 0.5	DBT0240 47.0 ± 0.5	DBT0027 47.3 ± 0.5

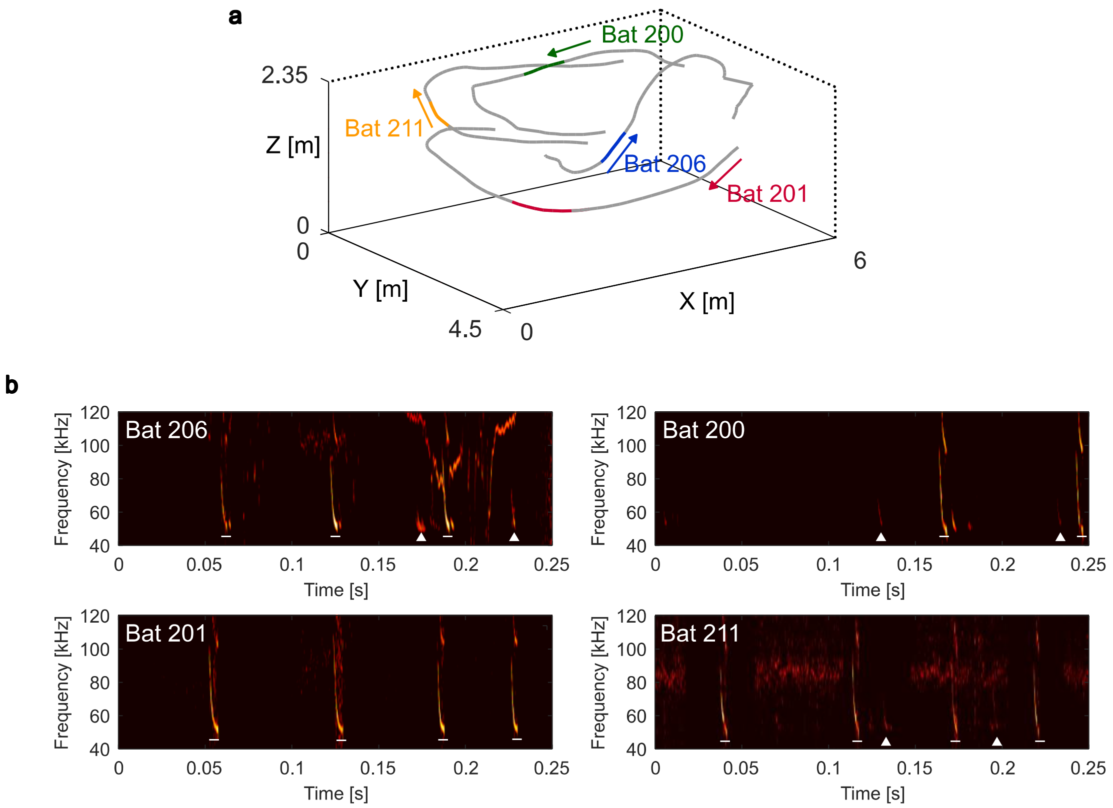


Figure 4-1 Echolocation behavior of four bats flying together. **a**, Flight trajectories of four simultaneously flying bats over a 5-s period. The colors indicate different bats (ID: 206, 201, 200, 211). The arrows indicate the flight directions. **b**, Spectrograms of pulses emitted by four bats during the 250-ms intervals indicated in a as colored lines on the trajectories. White bars indicate self-generated pulses, and white triangles indicate pulses from other bats.

Figures 4-2a-d show data from one specific group, presenting the flight trajectories and terminal frequencies of the pulses emitted by four bats during single flight 1 and group flight in a span of 5 s. Figures 4-2c and d show the terminal frequencies of echolocation pulses emitted by the bats during single and group flight as indicated by the trajectories in Figures 4-2a and b, respectively. Each bat flying in a group seemed to use a different terminal frequency (bat 222: 43.2 ± 0.6 kHz, bat 225: 45.3 ± 0.7 kHz, bat 226: 46.4 ± 0.5 kHz, and bat 229: 47.4 ± 0.9 kHz, Fig. 4-2c) whereas their terminal frequencies were similar during single flight (bat 222: 46.1 ± 0.6 kHz, bat 225: 46.7 ± 0.4 kHz, bat 226: 47.3 ± 0.5 kHz, and bat 229: 47.4 ± 0.7 kHz, Fig. 4-2d). Figure 4-2e shows the mean terminal frequencies of pulses emitted by bats in all six groups (19 bats in total) during single flight 1, group flight, and single flight 2. The bats shifted their terminal frequencies in both directions, upward and downward, in group flight compared with single flights 1 and 2. However, the mean terminal frequencies were not significantly different among flight conditions (one-way ANOVA, *P* = 0.365; the mean terminal frequencies were 47.1 ± 0.9 kHz, 47.1 ± 1.8 kHz, and 46.6 ± 0.9 kHz in single flight 1, group flight, and single flight 2, respectively). Next, we tested whether bidirectional changes in terminal frequency were caused by broadening of individual differences in terminal frequency. We defined Δterminal frequency as the difference in the mean terminal frequencies between the two bats that were closest in terms of their mean terminal frequencies. Figure 4-2f shows changes in the Δterminal frequencies among the three flight conditions for all groups. Bats flying in groups significantly increased their Δterminal frequencies from 0.6 ± 0.6 kHz in single flight 1 and 0.6 ± 0.4 kHz in single flight 2 to 1.1 ± 0.6 kHz in group flight (Tukey's HSD test, *P* < 0.05).

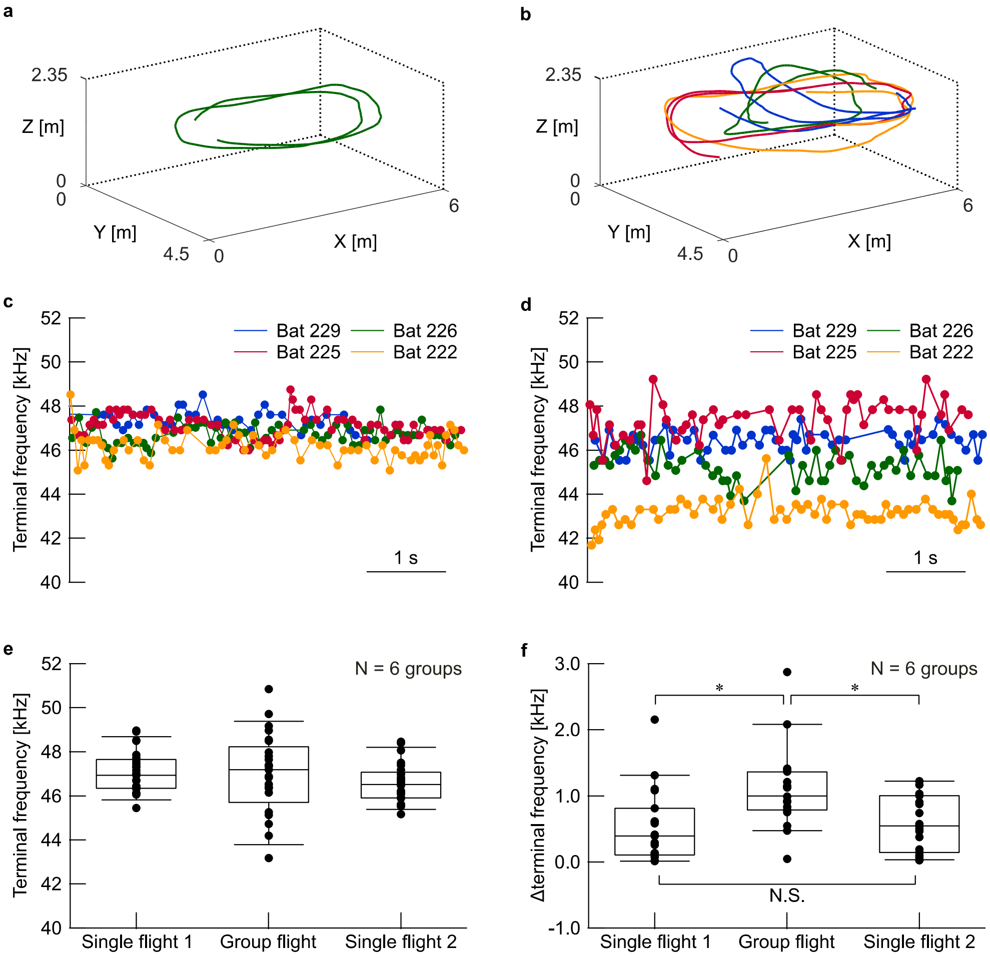


Figure 4-2 Echolocating bats use different terminal frequency channels during group flight. **a**, **b**, Flight trajectories of a bat during single flight 1 and four bats during group flight for group 1. **c**, **d**, Changes in the terminal frequencies of pulses emitted by four bats during single flight 1 and group flight. **e**, **f**, Changes in acoustic characteristics of pulses emitted by bats during single flight 1, single flight 2, and group flight. The horizontal lines inside the boxes show the medians. The upper and lower bounds of the boxes show first and third quartile, respectively. The horizontal bars above and below the boxes show the 10th and 90th percentiles, respectively. **e**, Mean terminal frequencies of each bat of all six groups in single flights 1, 2, and group flight. The data were collected from six groups (19 bats), and we obtained 24 data points per flight condition. **f**, Δterminal frequencies of all six groups in single flight 1, single flight 2, and group flight. We collected three Δterminal frequencies per group, which yielded 18 data points per flight condition. The mean terminal frequency did not differ among flight conditions (one-way ANOVA, $P = 0.365$). Δterminal frequencies were significantly increased in group flight compared with single flights 1 and 2 (Tukey's HSD test, $P < 0.05$).

On the other hand, acoustic characteristics other than terminal frequencies tended to simply increase during group flight compared with single flights 1 and 2 (Fig. 4-3). Start frequency (Fs) increased from 89.7 ± 5.4 kHz during single flight 1 and 89.3 ± 7.8 kHz during single flight 2 to 99.3 ± 6.0 kHz during group flight (Tukey's HSD test, $P < 0.05$; Fig. 4-3a). Bandwidth also increased from 43.3 ± 5.7 kHz during single flight 1 and 42.6 ± 7.9 kHz during single flight 2 to 52.1 ± 5.8 kHz during group flight (Tukey's HSD test, $P < 0.05$; Fig. 4-3b). The increase in Fs was much larger than any shifts in terminal frequencies, indicating that bandwidth was increased even when terminal frequencies was shifted upwards. Pulse duration increased from 3.2 ± 0.4 ms during single flight 1 and 3.0 ± 0.4 ms during single flight 2 to 3.8 ± 0.5 ms during group flight (Tukey's HSD test, $P < 0.05$; Fig. 4-3c). However, the interpulse interval did not significantly change. The mean IPIs were 82.4 ± 14.5 ms during single flight 1, 86.1 ± 19.4 ms during single flight 2, and 82.9 ± 14.4 ms during group flight (pne-way ANOVA, $P = 0.695$; Fig. 4-3d). No coordination of emission timing during group flight was observed (Fig. 4-4).

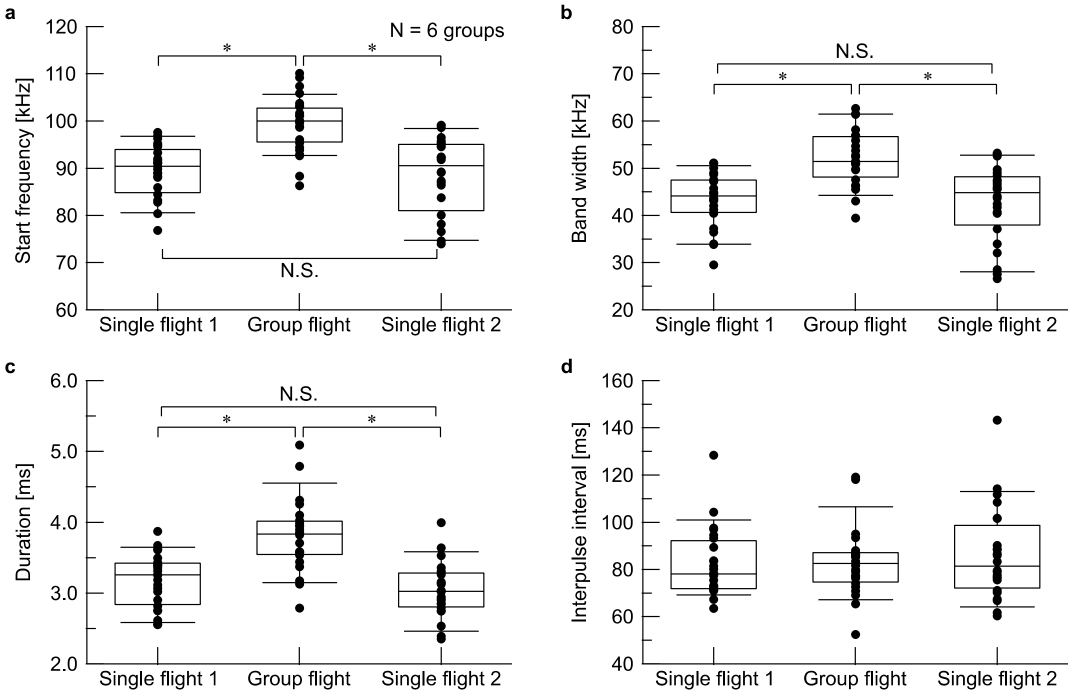


Figure 4-3 Means of the acoustic characteristics emitted by each bat in single flights 1 and 2 and group flight for all groups. **a**, Changes in Fs. **b**, Changes in duration. **c**, Changes in bandwidth. **d**, Changes in interpulse interval. We obtained 24 data points per flight condition. Horizontal lines inside boxes show medians. The upper and lower bounds of the boxes show first and third quartile, respectively. The horizontal bars above and below the boxes show the 10th and 90th percentiles, respectively. Fs, bandwidth, and duration were significantly increased from single flights 1 and 2 to group flight (Tukey's HSD test, $P < 0.05$). On the other hand, IPIs did not significantly differ among flight conditions (one-way ANOVA, $P = 0.695$).

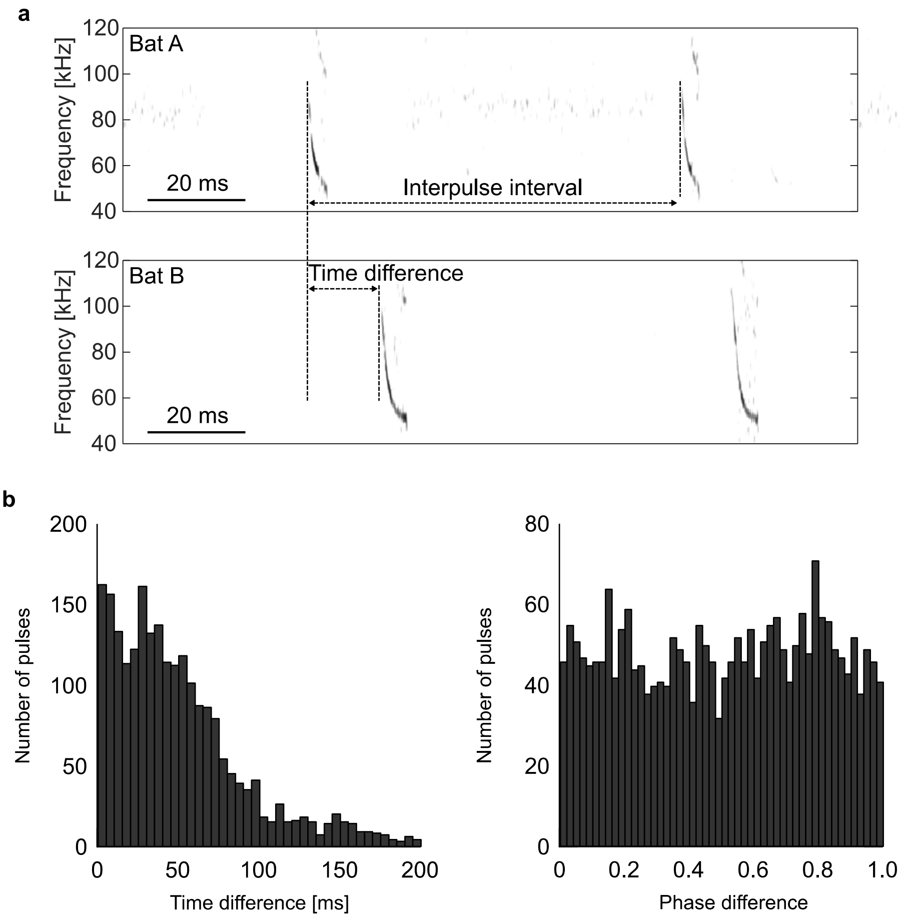


Figure 4-4 Emission timing of echolocation pulses during group flight. **a**, Definitions of the time difference and phase difference. The time difference is the difference between the emission time of one bat and those of other bats. The phase difference is obtained by dividing the time difference by the interpulse interval. We calculated the time difference and the phase difference of each possible pair of bats in each of the six groups. **b**, Histograms of time difference (left) and phase difference (right).

4. 2. 2. Similarity of bat-like signals with acoustic manipulations

We explored how changes in acoustic characteristics affected the similarities among bat echolocation pulses. First, to confirm that the similarity of echolocation pulses was lower in group flight than in single flight, we calculated the cross-correlations of pulses between individuals in a group when they flew singly and in the group. Cross-correlation was applied to the time-series amplitude waveforms of echolocation pulses after their amplitudes were normalized. This procedure was carried out on all the pulses that were used for sound analysis. Figure 4-5a shows representative echolocation pulses emitted by four bats (pulses A, B, C and D, respectively) during group flight, as well as the correlation values between pulse A and pulses A, B, C, and D normalized to the peak autocorrelation value of pulse A. The peak cross-correlation values with pulse A were 0.19 (vs. pulse B), 0.38 (vs. pulse C), and 0.34 (vs. pulse D) (Fig. 4-5a, bottom). We defined the similarity index between two bats as the peak value of the cross-correlation of their pulses normalized to the autocorrelations of pulses of either bat (see Methods). Figure 4-5b shows that the similarity indices between individuals were significantly lower in group flight (0.21 ± 0.07) than in single flight 1 (0.32 ± 0.12) or single flight 2 (0.29 ± 0.12) (Tukey's HSD test, $P < 0.05$).

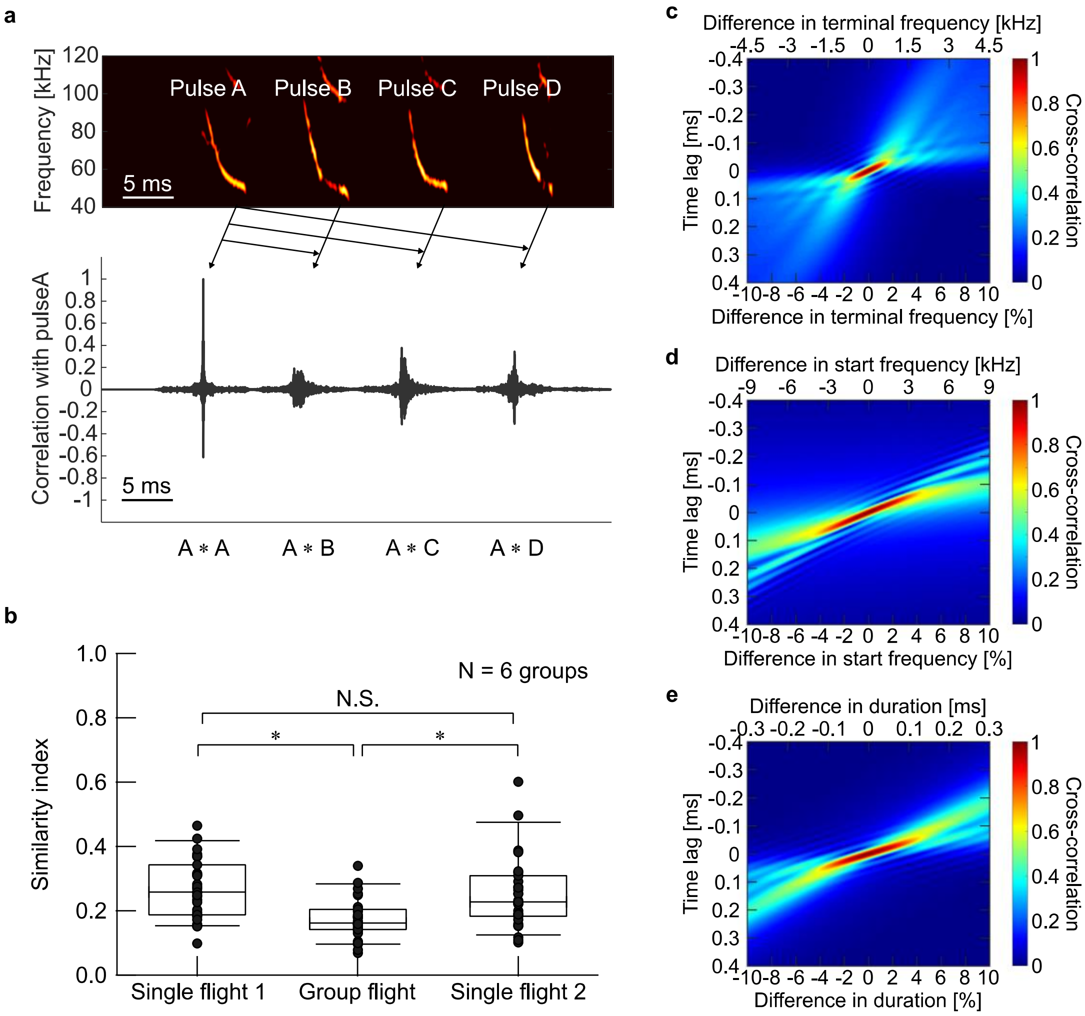


Figure 4-5 Similarities between pulses among bats of the same group and the effects of changes in acoustic characteristics on the similarities between frequency-modulated signals. **a**, Spectrograms of the pulses of four bats flying together (top); correlation values of pulses A, B, C, and D with pulse A (bottom). **b**, Peak cross-correlation values normalized to the autocorrelation values. We analyzed the similarity indices of all pairs of calls between four individuals in each group. This analysis yielded 36 data points per flight condition (see Methods). The upper and lower bounds of the boxes show first and third quartile, respectively. The horizontal bars above and below the boxes show the 10th and 90th percentiles, respectively. The peaks (similarity indices) significantly decreased in group flight compared with single flights 1 and 2 (Tukey's post hoc test, *P* < 0.05). **c-e**, Changes in the dissimilarity function when the cross-correlation was calculated between the original frequency-modulated signal (90-45 kHz downward with a duration of 3 ms) and the manipulated signal in terms of (**c**) Fs, (**d**) terminal frequency, and (**e**) duration from -10% to 10%

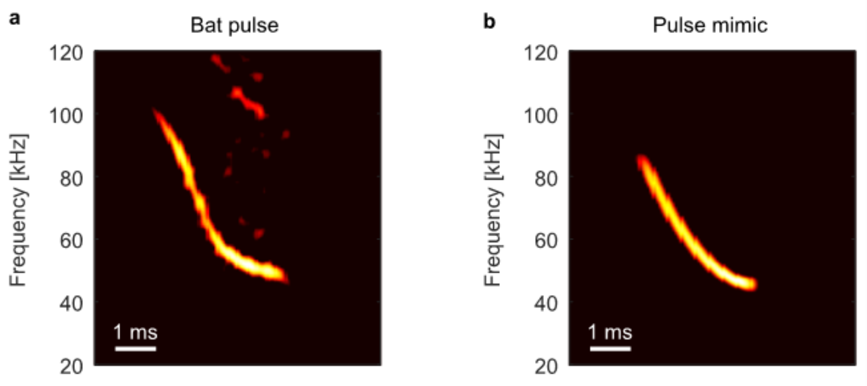


Figure 4-6 Typical echolocation pulse of *M. fuliginosus* and pulse mimic. **a,** Spectrogram of a typical echolocation pulse emitted by *M. fuliginosus* recorded with a Telemike. **b**, Spectrogram of a pulse mimic for calculation of the dissimilarity function. The generated pulse had an Fs of 90 kHz, a terminal frequency of 45 kHz, and a duration of 3 ms.

Next, we examined which acoustic characteristics most affected similarity. We prepared a frequency-modulated signal that mimicked the echolocation pulses of *M. fuliginosus*, with an Fs of 90 kHz, a terminal frequency of 45 kHz, a bandwidth of 45 kHz, and a duration of 3 ms (Fig. 4-6). Then, we calculated dissimilarity functions (i.e., cross-correlations between the signal and an acoustically modified version of the signal [in terms of Fs, terminal frequency, and duration, which we gradually changed from -10% to 10%]; see Methods). The cross-correlation values fell most when the terminal frequency of the signal was manipulated, although changes in other characteristics also resulted in decreases in cross-correlation values. Figure 4-5c shows that the half-width at half-maximum of the dissimilarity function was obtained when the terminal frequencies was changed by only 2%, corresponding to approximately 1 kHz (the mean terminal frequencies was approximately 48 kHz). The half-width at half-maximum was obtained when Fs was changed by 9% (corresponding to approximately 8 kHz) and the duration was changed by 7% (corresponding to approximately 0.2 ms) (Figs. 4-5d and e).

4.3. Discussion

In this study, we recorded separate echolocation sounds from bats flying together in groups of four; we found that individuals shift their terminal frequencies away from those of conspecifics. The bats changed the acoustic features of their pulses when flying with multiple conspecifics. Specifically, bidirectional changes in terminal frequencies significantly broadened the differences in terminal frequency among group members (Fig. 4-2e). Although the direction of change in terminal frequency was almost constant within individuals, there were some exceptions (Fig. 4-7). There was a tendency for individuals with lower terminal frequency to shift much lower in group flight and vice versa (Fig. 4-8). Similarities in echolocation pulses among individuals were significantly decreased during group flight in comparison with single flights 1 and 2. In addition, shifts in terminal frequency were much more helpful than shifts in other acoustic features for differentiating the signal from other signals with similar characteristics, as revealed by computation of cross-correlations between a frequency-modulated signal mimicking bat pulses ('original') and a copy in which an acoustic feature was changed ('manipulated') (Fig. 4-5c). These results show that echolocating bats enhance the individual features of emitted pulses ("identities") to solve the problem of signal jamming, as do other animals (weakly electric fish) that use active sensing in a different sensory modality.

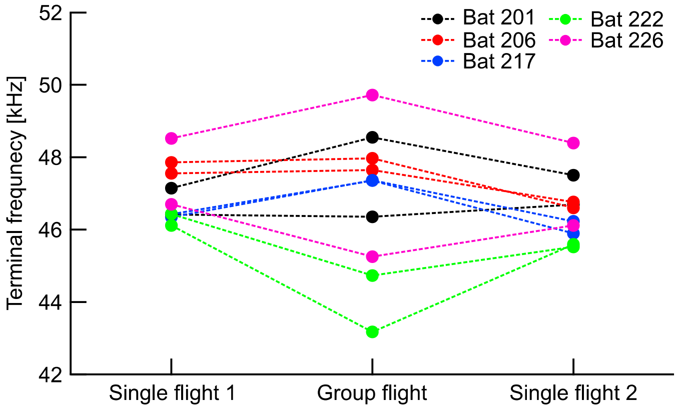


Figure 4-7 Directions of changes in terminal frequency by the same bats in different group flights. Changes in the mean terminal frequency of the bats that experienced group flight twice in single flight 1, group flight, and single flight 2. Different colors indicate different bats. Although the bats tended to shift their terminal frequency in the same directions in two different group flights, there were some exceptions (e.g., Bat 201 and Bat 226).

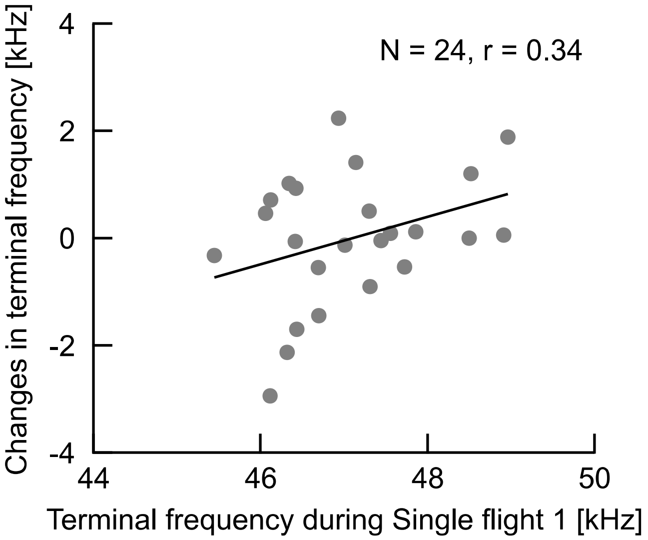


Figure 4-8 Relationship between changes in terminal frequency and terminal frequency during single flight 1. The plot shows changes in mean terminal frequency and mean terminal frequency during single flight 1. The solid line is a regression line. Individuals with lower terminal frequency shifted much lower in group flight, and vice versa. There was a weak correlation between mean terminal frequencies during single flight 1 and changes in terminal frequency from single flight 1 to group flight (r = 0.34).

Eptesicus fuscus bats shift their terminal frequencies upward or downward away from the frequencies of constant-frequency (CF) jamming sounds when the jamming frequencies are close to the terminal frequencies (M. E. Bates et al., 2008). Similarly, flying frequency-modulating bats shifted their terminal frequencies when flying in pairs or in the presence of frequency-modulated jamming sounds (C. Chiu et al., 2009; E. H. Gillam et al., 2007; K. Hase et al., 2016; E. Takahashi et al., 2014; N. Ulanovsky et al., 2004). In the present study, we observed that differences in the terminal frequencies of pulses emitted by bats during group flight were significantly greater than in single flight, and the similarities among pulses also significantly decreased in group flight compared to single flight. Taken together, the findings suggest that echolocating bats maintain or expand differences in frequency because it is important for bats to avoid spectral jamming by other conspecifics.

On the other hand, some recent studies have cast doubt on the notion that the JAR involves shifting of frequencies (E. Amichai et al., 2015; N. Cvikel et al., 2015b; S. Götze et al., 2016). These studies suggest that the spectral shifts are caused by the physical presence of nearby individuals, rather than acoustic jamming from conspecifics. The JAR is suggested not to be attributable to shifting of frequencies, as most previous studies focused only on changes in the frequencies of emitted pulses and not on increased inter-individual differences in frequency (corresponding to the Δterminal frequencies of the present study). In the present study, there was no significant correlation between terminal frequency and the duration of emitted pulses, indicating that the observed changes in terminal frequency during group flight were not due to the changes in duration caused by the changes in distance from other individual bats flying in the group (Fig. 4-9). In addition, the present study was the first to track and identify pulses emitted by each individual in groups of four bats without distortion. As a result, we observed bidirectional changes in terminal frequencies and decreases in the similarity of pulses among individuals during group flight; we tested the same bats in group flight and single flights scheduled before and after the group flights (Figs. 4-2e and f). We found that the Δterminal frequencies were smaller (approximately 1 kHz) than the inter-individual differences or flight-induced Doppler shifts (approximately 0.5 to 1.5 kHz in this study); thus, they may have been overlooked in previous studies because of the methodologies used.

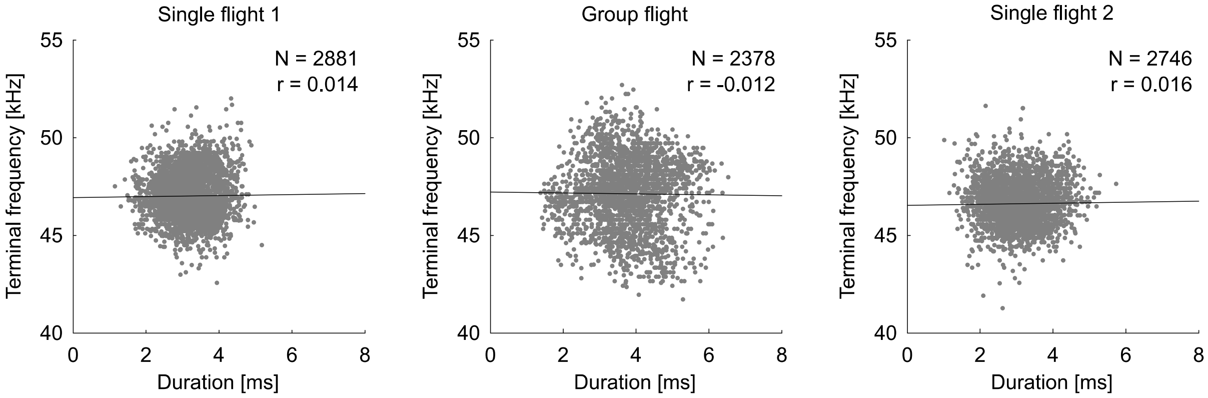


Figure 4-9 Correlation of terminal frequency with duration of emitted pulses. Black lines indicate regression lines. The data did not show a correlation between terminal frequency and duration in single flight 1 (left), group flight (middle), or single flight 2 (right), indicating that the observed changes in terminal frequency were not due to the changes in duration caused by the changes in distance from other individual bats flying in the group.

The echolocation pulses emitted by some species of frequency-modulating bats (including *M. fuliginosus*, *Pipistrellus abramus*, and *E. fuscus*) are composed of a frequency-modulated portion that is specialized for measuring distance and a quasi-CF (QCF) portion that is helpful for target detection. The bats may be able to use both aspects of compound frequency-modulated-QCF pulses to measure target distances and detect relatively distant targets. We found that even a slight difference in the terminal frequency of bat-like signals reduced the similarity between sounds. Therefore, the frequency of the QCF portion may serve as a “tag” that indicates the identity of an individual pulse and may be used by bats to discriminate their own echoes from those of others.

We also observed increases in sound intensity and peak frequency during group flight (Fig. 4-10). It has been reported that bats confronted with noises increase the sound intensity and/or frequency of their emitted pulses (E. Amichai et al., 2015; S. R. Hage et al., 2013). The involuntary regulation of the intensity, pitch, and/or syllable duration of vocalizations in animals, including humans, in the presence of noise is called the Lombard effect (H. Brumm & D. Todt, 2002; H. Brumm et al., 2004; M. Garnier et al., 2010; S. Roy et al., 2011; W. V. Summers et al., 1988). The increase in the intensity of emitted pulses and the lengthening of pulse duration, which increase the concentration of energy in the low frequency range in the outgoing signals, may result in an improved signal-to-noise ratio. Moreover, longer sounds are more detectable than shorter sounds because the auditory system can integrate sound over time (P. Heil & H. Neubauer, 2003). Our results suggested that echolocating bats emit louder and longer pulses in noisy situations to improve the signal-to-noise ratio of their returning echoes, as suggested by previous studies (E. Amichai et al., 2015; S. R. Hage et al., 2013).

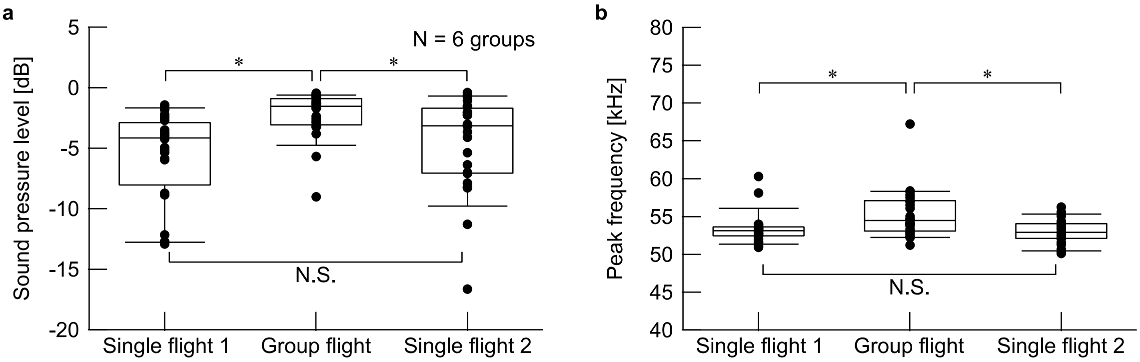


Figure 4-10 Mean acoustic characteristics of pulses emitted by each bat in each group in single flights 1 and 2 and in group flight. **a**, Changes in SPL. **b**, Changes in peak frequency. SPL was calculated from the peak-to peak-amplitude voltage of each pulse in the time domain. A value of 0 dB was defined as the maximum value of pulses recorded among all flight conditions. We plotted 24 data points per flight condition. The horizontal lines inside the boxes show the medians. The upper and lower margins of the boxes show the first and third quartiles, respectively. The horizontal bars above and below the boxes show the 10th and 90th percentiles, respectively. SPL and peak frequency were significantly increased from single flights 1 and 2 to group flight (Tukey's HSD test, $P < 0.05$).

How do our current results apply to group flight of bats in the real world? Although shifts in frequency are helpful to avoid acoustic jamming, the shifts cannot fully explain the ability of bats to fly with enormous numbers of conspecifics in nature. Echolocating bats can use other potential solutions to avoid acoustic interference from other bats. Vocal timing is one of the most effective means to avoid confusion, especially in low-duty-cycle bats. Stationary *T. brasiliensis* reduce the number of pulses in the presence of interfering sounds and conspecifics (J. Jarvis et al., 2010; J. Jarvis et al., 2013). Similarly, flying *P. abramus* (E. Takahashi et al., 2014), *T. brasiliensis* (A. M. Adams et al., 2017) and *E. fuscus* (C. Chiu et al., 2008) bats regulate vocal timing in response to jamming sounds or the sounds of conspecifics in the group. Moreover, the directionality and directivity of the ears and pulses serve as spatial filters, allowing bats to focus on a point in three-dimensional space and ignore sounds that come from off-axis angles.

Echolocating bats may use these mechanisms effectively to avoid jamming by the pulses emitted from other bats, allowing effective collective behavior when groups contain large numbers of bats. Our experimental design focused on addressing the mechanisms of jamming avoidance for small groups of bats flying in echoic closed spaces. It seems difficult for each bat to find an “open slot” through terminal frequency alone when a swarm of dozens of flying bats results in a chaotic acoustic environment.

We are the first to show directly that groups of bats mutually separate their frequencies to reduce the similarities between pulses of different individuals. On the basis of previous work on the JAR of electric fish, which increase frequency differences when in self-generated electric fields (T. H. Bullock et al., 1975), we suggest that animals using active sensing employ universal rules that transcend species and sensory modality boundaries. Furthermore, our calculations show that bat-like combination signals (with frequency-modulation and QCF portions) can be differentiated by slight shifts in frequency. Although it is currently difficult to correlate the results of our computations with auditory perception in bats, this simple strategy could also be used as a method of signal separation in various engineering fields, including radar or sonar research.

4.4. Materials and Methods

4. 4. 1. Subjects

We used 19 *M. fuliginosus* bats (body mass, 12.6–18.1 g; 10 males and 9 females) in this study. We collected the bats from large colonies roosting in natural caves in Hyogo and Fukui prefectures, Japan. We were licensed to collect the bats, and we complied with all Japanese laws (permits from Hyogo prefecture in 2015 and from Fukui prefecture in 2016 and 2017). The animals were housed in a temperature- and humidity-controlled colony room [4 (L) × 3 (W) × 2 m (H)] at Doshisha University in Kyoto, Japan. They were allowed to fly freely and had ad libitum access to food (mealworms) and vitamin-enriched water. The day-night cycle of the room was set to 12 h:12 h dark: light.

All experiments complied with the Principles of Animal Care (publication no. 86-23 [revised 1985]) of the National Institutes of Health) and all Japanese laws. All experiments were approved by the Animal Experiment Committee of Doshisha University.

4. 4. 2. Experimental procedure

All flight experiments were conducted in an experimental chamber [9 (L) × 4.5 (W) × 2.4 m (H)] at Doshisha University in Kyoto, Japan. The chamber was constructed of steel plates to minimize interference from external electromagnetic noise and commercial frequency-modulation radio stations. There was no acoustic foam because we wanted to make the chamber more echoic and make the acoustic situation more extreme in order to elicit clear jamming avoidance behavior. During all experiments, long-wavelength lighting with filters (removing wavelengths below 650 nm) was used to avoid visual effects on the bats. Nineteen bats were randomly assigned to six groups of four bats, with some overlap (Table 4-1). The bats were allowed to fly individually and in groups in a flight space [6 (L) × 4.5 (W) × 2.4 m (H)] surrounded by walls and a net suspended from the ceiling within the experimental chamber. There were no obvious landing sites in the flight space.

The bats were tested under three experimental conditions: single flight 1, group flight, and single flight 2. For each group, all flights were conducted within one day. The detailed procedure was as follows. First, each bat in a group was released by an experimenter and flew individually in the experimental chamber for approximately 30 s (single flight 1). After those single flights were recorded, each bat was kept in an individual cage. Then, two experimenters released four bats simultaneously so that they flew together in the chamber for approximately 60 s (group flight). Finally, once more, an experimenter allowed each bat in the group to fly alone in the chamber for approximately 30 s (single flight 2).

4. 4. 3. Telemike recordings

Echolocation pulses emitted by each flying bat were recorded by a custom-made miniature on-board microphone (Telemike) mounted on the back of the bat. The details of the Telemike recording procedure have been described previously (S. Hiryu et al., 2007). To record separately the echolocation pulses emitted by each bat flying in a group, we attached a Telemike to the back of each individual bat. The Telemike transmitted frequency-modulation radio signals using a carrier frequency between 76 and 104 MHz.

We assigned a different carrier frequency to each Telemike within a group so that the transmitted signals would not interfere with each other. After the transmitted signals had been received by a frequency-modulation radio antenna (Terk Technologies Corporation, FM+, Commack, New York, USA) suspended from the ceiling of the chamber, they were demodulated using a custom-made frequency-modulation receiver (ArumoTech Corporation, Kyoto, Japan) featuring five independent channels with bandpass filters of 10–200 kHz. The signals were then digitized using a high-speed data-acquisition card (National Instruments, Model NI PXI-6358, Tokyo, Japan, 16 bit, $f_s = 500$ kHz). The total frequency response of the Telemike system was flat (within ±3 dB) between 20 and 100 kHz.

4. 4. 4. Video recordings

Video recordings were made by two digital video cameras (IDT Japan, Inc., MotionXtra NX8-S1, Tokyo, Japan) running at 30 frames per second. The cameras were located outside the flight space (at two of the top corners of the chamber). The captured video images were stored on a personal computer. The two video cameras recorded a three-dimensional cube of known coordinates positioned in the center of the fw space before the flight experiments commenced. Three-dimensional reconstruction of each bat's flight path was performed with motion capture software (Ditect Corporation, DippMotion PRO version 2.21a, Tokyo, Japan) using direct linear transformation with reference to the coordinates of the reference frame.

4. 4. 5. Sound analysis

The number of pulses we analyzed for each bat ranged from 173 to 467. The pulse counts were different because we analyzed only those pulses that occurred when the four bats were actually flying together and the telemetry recordings had a good signal-to-noise ratio. We also excluded pulses before and after the buzz because those pulses sometimes had unusual duration and terminal frequency (they were much shorter and had lower terminal frequency). As a result, we analyzed 4.2 to 9.8 seconds (mean duration of 6.5 seconds), depending on the duration of time the bats spent flying in groups of four. The acoustic characteristics of echolocation pulses were manually analyzed on spectrograms from Telemike recordings using custom-written MATLAB scripts running on a personal computer. Each Telemike was intended to record the echolocation pulses and echoes of one bat. However, during group flight, the microphones sometimes recorded not only the pulses of the intended bat but also those of other bats. To extract and analyze the echolocation pulses of individual bats correctly in such cases, we visually discriminated pulses on the basis of amplitude and timing across oscillograms and spectrograms of the four recorded channels.

We defined the Fs and terminal frequency of each sound as the highest and lowest frequencies, respectively, of each pulse in the spectrogram that lay -25 dB from the maximum energy portion of the spectrogram. The duration was also determined from the spectrogram, at -25 dB relative to the maximum energy portion. The bandwidth (BW) was calculated by subtracting the terminal frequency from the Fs. We defined a neighboring bat as the bat with the nearest mean terminal frequency to a bat in the same group. Δterminal frequency was defined as the difference in mean terminal frequency between a bat and the neighboring bat.

All statistical analyses were performed using SPSS version 24 (IBM, Armonk, New York, USA). We employed one-way ANOVA to test whether the terminal frequency, Δterminal frequency, Fs, bandwidth, duration, interpulse interval, or cross-correlation peak values differed significantly among the three flight conditions (single flight 1, group flight, and single flight 2). If the main effect was significant, we then applied Tukey's post hoc test. *P*-values < 0.05 were considered significant. The results are presented as the means ± SDs.

4. 4. 6. Similarity index

To explore whether the similarities of pulses among individuals in a group changed significantly, we used cross-correlation as a similarity index. Cross-correlation values were calculated between the pulses of each pair of individuals in each group, creating six combinations per group (${}_4C_2$ per group). Cross-correlation was applied to the time-series amplitude waveforms of echolocation pulses after their amplitudes were normalized. This procedure was carried out on all the pulses that were used for sound analysis. For example, to obtain the similarity index between bat A and bat B in single flight 1, we conducted cross-correlation for all pairs of calls between bat A and bat B during the flight (for example, bat A and bat B of group 1 produced 128 and 152 pulses, respectively). The obtained cross-correlations of any combination were normalized to the autocorrelation values of the pulses of either bat. We defined the similarity index as the mean of the peak values of the normalized cross-correlations between two individuals. This analysis yielded 36 similarity indices (${}_4C_2$ combinations × six groups) per flight condition (single flight 1, group flight, and single flight 2).

4. 4. 7. Dissimilarity function

We created frequency-modulated signals modulated from 90 to 45 kHz over a 3-ms period, mimicking the pulses of *M. fuliginosus*. We calculated cross-correlations between the original signals and the acoustically modified signals when each acoustic characteristic (Fs, terminal frequency, and duration) was gradually changed from -10 to 10%. The calculated cross-correlation values were plotted as functions of the modifications in the acoustic features. The half-width at half-maximum values of the dissimilarity functions showed how changes in an acoustical feature affected the degree of similarity between the two signals.

Chapter 5: Group-flying horseshoe bats make adaptive changes in pulse characteristics but not in echo frequency: Doppler shift compensation facilitates weak echo extraction under jamming

Some species of echolocating bats utilize compound sounds consisting of constant-frequency (CF) and frequency-modulated (FM) components for orientation and hunting. They control CF frequency of emitted pulses to stabilize echoes CF frequency within a narrow frequency range to which their auditory sensitivity is extremely high; this behavior is called Doppler shift compensation. Groups of conspecifics may hunt together, leading to an acoustically complex scenario, but it is not fully understood how signal jamming is avoided. In the present study, echolocation pulses and returning echoes when *Rhinolophus ferrumequinum nippon* flew alone or in a group of three bat were compared in spaces of differing largeness (wide and narrow spaces). We found that group-flying bats in the narrow space increased the duration and bandwidth of the terminal FM (tFM) component of their vocalizations. Group-flying bats also decreased the duration of CF–FM pulses and increased the silent interval between pulses. These changes in echolocation behavior may be an adaptation to avoid signal jamming during group flight. In contrast, the frequency of the returning echoes did not differ in the presence of conspecifics. We estimated the received frequency of the pulses emitted by individuals and found that their own echo frequencies were compensated within narrow frequency ranges by Doppler shift compensation, whereas the frequencies of the received pulses emitted by other bats were much more broadly distributed. Our results suggest that the bats' auditory systems are sharply tuned to a narrow frequency to filter spectral interference from other bats.

5.1. Introduction

Echolocation is a form of active sensing that allows bats to navigate through dark environments. By manipulating the timing of ultrasound emissions, they can capture a small prey item without vision. The emission rate is increased as they approach their prey to keep it within their acoustic field of view (N. Matsuta et al., 2013). The ability to control sensory acquisition timing is one of the greatest advantages of active sensing. However, individuals are exposed to conspecific signals when echolocating in groups (A. Surlykke & C. F. Moss, 2000). How echolocating bats mitigate signal jamming is one of biggest questions in echolocation research.

A number of studies have demonstrated frequency-shifting jamming-avoidance behavior in frequency-modulating (FM) bats, where bats change their echolocation frequency to avoid spectral overlap during group flying (C. Chiu et al., 2009; J. Habersetzer, 1981; K. Hase et al., 2018; M. K. Obrist, 1995; J. M. Ratcliffe et al., 2004; N. Ulanovsky et al., 2004) or when they are exposed to noises (M. E. Bates et al., 2008; E. H. Gillam & B. K. Montero, 2015; E. H. Gillam et al., 2007; K. Hase et al., 2016; E. Takahashi et al., 2014). *Miniopterus fuliginosus* flying in a group of four bats shifted the terminal frequency of their emitted pulses away from each other to mitigate signal jamming from other bats (K. Hase et al., 2018). However, to our knowledge only one study has measured bat echoes during group flight (Y. Furusawa et al., 2012), allowing a closer look at how their auditory system process weak echoes in noisy situations.

Some species of bats from the Rhinolophidae, Hipposideridae, and Mormoopidae families use multifaceted constant-frequency (CF) and FM sounds and change their pulse frequency to compensate for frequency shifts that occur in their echoes due to the Doppler effect (S. Hiryu et al., 2016). This behavior, called Doppler shift compensation, is thought to be an adaptation to register echoes within their optimal frequency range, or reference frequency, as an aid in detecting insects in cluttered environments where echoes reflect off surfaces such as leaves (H.-U. Schnitzler & A. Denzinger, 2011). Their auditory system is highly specialized to detect an extremely narrow frequency range, called auditory fovea.

In the presence of conspecific or heterospecific bats, *Rhinolophus capennsis* changed the duration of their FM component and bandwidth of their CF–FM pulses, but not the CF frequency, compared to when they flew alone (K. Fawcett et al., 2015). Similarly, G. Jones et al. (1994) found no systematic changes in pulse frequency in *Hipposideros* in the presence of the playback. On the other hand, Y. Furusawa et al. (2012) demonstrated that *Rhinolophus ferrumequinum nippon* flying in pairs became more similar in their reference frequencies when flying together; this paradoxical shift may function to allow as many bats as possible within the reference frequency. The aforementioned studies suggested that vocalizations from other bats can be filtered out by the auditory fovea. However, these studies did not assess the frequency of pulses of other bats received by one bat that can change due to the Doppler effect and the amount of doppler shift compensation. In the present study, we compared echolocation pulses and their echoes between group and single flight and between flight spaces of different largeness by attaching on-board telemetry microphones to individuals. Using the telemetry microphones, we also investigated the frequency of their echoes compared to the frequency of received pulses coming from other bats in the group estimated from the relative velocity between individuals.

5.2. Materials and Methods

5. 2. 1. Subjects

Adult Japanese horseshoe bats (*Rhinolophus ferrumequinum nippon*, n = 9, 6 males and 3 females) were caught from wild colonies within natural caves in Fukui Prefecture, Japan. All collections were conducted in compliance with Japanese law for bat collection under permits received from Fukui Prefecture and Doshisha University.

The echolocation sounds emitted by the bats consist of a relatively long CF portion, an accompanying brief upward initial FM (iFM) component and a brief downward terminal FM (tFM) component. The bats emit multi-harmonic echolocation sounds, the second harmonic of CF components (CF2) is the most prominent. The iFM component is often very weak or absent. The bats exhibit Doppler shift compensation behavior to compensate for the echo CF2 frequency, which varies between individuals.

5. 2. 2. Experimental procedure

All experiments were conducted in an experimental chamber (9 × 4.5 × 2.4 m) at Doshisha University in Kyoto, Japan. The chamber was constructed of steel plates to mitigate interference from external electromagnetic noise and commercial FM radio stations. To assess how reference frequency changed during group flight, no acoustic foam was used to ensure that the chamber was echoic. To investigate how acoustic interferences affected echolocation behavior, we created flight spaces that varied in largeness. The wide chamber (6 × 4.5 × 2.4 m) was constructed of a single net suspended from the ceiling 6 m from the front wall (Fig. 5-1a). The narrow chamber (2 × 4.5 × 2.4 m) was constructed by adding a suspended net to the wide chamber (Fig. 5-1b). An onboard telemetry microphone was attached to the back of each bat in a group to separately record emitted pulses and returning echoes belonging to all individuals flying together. During all experiments, a long-wavelength light with filters (removing wavelengths below 650 nm) was used to minimize visual effects on the bats.

We randomly assigned nine bats to 12 groups consisting of three bats, with some overlap. The bats were tested under three experimental conditions: single flight1, group flight, and single flight 2. Some groups (six groups) were tested in the wide space, and the others (six groups) were tested in the narrow space.

5. 2. 3. Telemike recordings

Echolocation pulses and echoes for each individual were separately recorded by a custom-made miniature on-board microphone (Telemike) mounted on the bat’s back. The detailed procedure for recording sounds of multiple bats has been described previously (Hase et al. 2018). The Telemike transmits FM radio signals using a carrier frequency between 76 and 104 MHz. A different carrier frequency was assigned to each Telemike within a group so that the transmitted signals would not interfere with each other. After the signals had been received by an FM radio antenna (Terk Technologies Corporation, FM+, Commack, New York, USA) suspended from the ceiling of the chamber, they were demodulated using a custom-made FM receiver (ArumoTech Corporation, Kyoto, Japan) with bandpass filters of 10–200 kHz. The signals were then digitized using a high-speed data-acquisition card (National Instruments, Model NI PXI-

6358, Tokyo, Japan, 16 bit, f_s = 500 kHz).

5. 2. 4. Video recordings

A three-dimensional flight trajectory was calculated for each bat by recording flight using two digital video cameras (30 fps; IDT Japan, Inc., MotionXtra NX8-S1, Tokyo, Japan). The cameras were located outside the flight space at the top corners of the chambers. The video images were analyzed using motion capture software (DippMotion PRO version 2.21a, Ditect Corporation, Tokyo, Japan). Three-dimensional position coordinates of each bat were calculated by the direct liner transformation (DLT) method, which derives the bat's position from the parallax of camera images from two directions. Images captured with the video cameras and sounds measured using Telemike were synchronized with the trigger signal manually generated by an experimenter.

5. 2. 5. Analysis

The echolocation pulses and echoes were manually analyzed from the Telemike recordings on spectrograms using custom-written MATLAB scripts. Pulse duration, bandwidths, and tFM duration were determined from the spectrogram. The CF2 frequencies of pulses and echoes were calculated from a visually selected area around the CF component by a fast Fourier transformation of over 16,384 sample points. This analysis resulted in a frequency resolution of 31 Hz. We defined the silent time as the interval between sonar sound groups, which are grouped pulses emitted in relatively short interpulse intervals (IPIs).

During group flight, Telemikes sometimes recorded pulses of other individuals besides the focal bat. To extract the echolocation pulses of the focal bat, visual discrimination was used based on the power and timing across spectrograms of the three recorded channels.

We tested whether acoustic characteristics of echolocation pulses and echoes were affected by group flight compared with single flight using one-way ANOVA. If the main effect was significant, we then applied Tukey's post hoc test. $P < 0.05$ was considered significant. We used SPSS version 24 (IBM, Armonk, New York, USA) for all statistical analyses. Results are presented as mean ± standard deviation (SD).

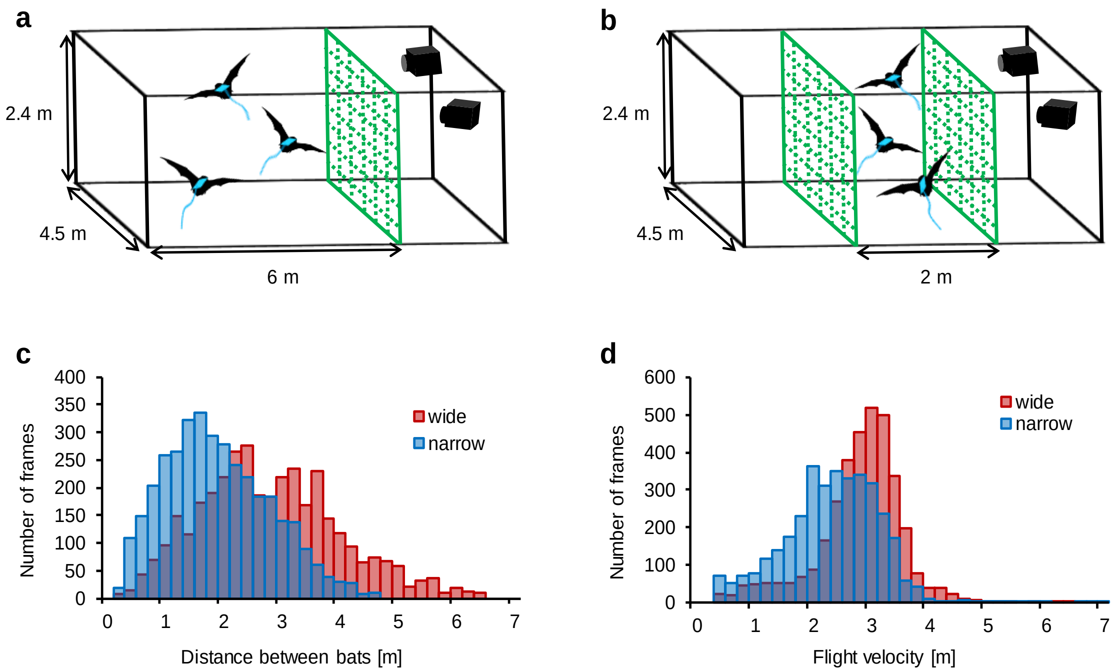


Figure 5-1 Two types of flight spaces; the wide space (a) and the narrow space (b). Differences in distance between bats (c) and flight velocity (d) during group flight in the wide and narrow spaces.

5.3. Results

5. 3. 1. Changes in acoustic characteristics of echolocation pulses

The inter-individual distances and flight speed of Japanese horseshoe bats in group flight were different between wide and narrow spaces, indicating that we could construct different clutter levels (Fig. 5-1c and d). The mean distance between bats during group flight was shorter in the narrow (1.8 ± 0.9 m) than in the wide space (2.7 ± 1.2 m; Fig. 5-1c). The mean flight velocity was lower in the narrow space (2.2 ± 0.8 m/s) than the wide space (2.7 ± 0.7 m/s; Fig. 5-11d).

Figure 5-2 shows representative flight trajectories, spectrograms of emitted pulses, and returning echoes recorded with a Telemike carried by each individual. The Telemikes recorded pulses and echoes of other bats in addition to those of the focal individual, suggesting sound transfer during group flight.

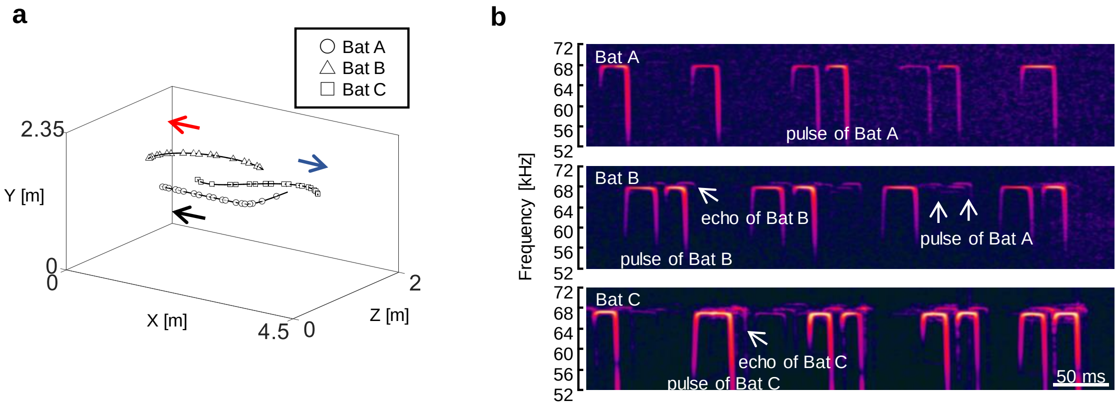


Figure 5-2 Representative flight trajectories (a) and spectrograms (b) of three bats flying together.

The bandwidth of the tFM component (tFM bandwidth) was not significantly different among single flight 1 (14.1 ± 2.4 kHz), single flight 2 (13.5 ± 2.1 kHz), and group flight (14.6 ± 2.3 kHz) in the wide space (Fig. 5-3a, one-way ANOVA, $p = 0.339$). On the other hand, tFM bandwidth was significantly changed from single flight 1 (13.5 ± 1.8 kHz) and single flight 2 (13.8 ± 2.1 kHz) to group flight (16.3 ± 2.0 kHz) in the wide space (Fig. 5-3a, Tukey's HSD test, $p < 0.05$). Moreover, the duration of the tFM component (tFM duration) was not significantly different among single flight 1 (2.0 ± 0.3 ms), single flight 2 (2.0 ± 0.4 ms), and group flight (2.3 ± 0.4 ms) in the wide space (Fig. 5-3b, one-way ANOVA, $p = 0.08$). tFM duration was significantly changed from single flight 1 (1.6 ± 0.2 ms) and single flight 2 (1.6 ± 0.2 ms) to group flight (1.9 ± 0.2 ms) in the narrow space (Fig. 5-3b, Tukey's HSD test, $p < 0.05$).

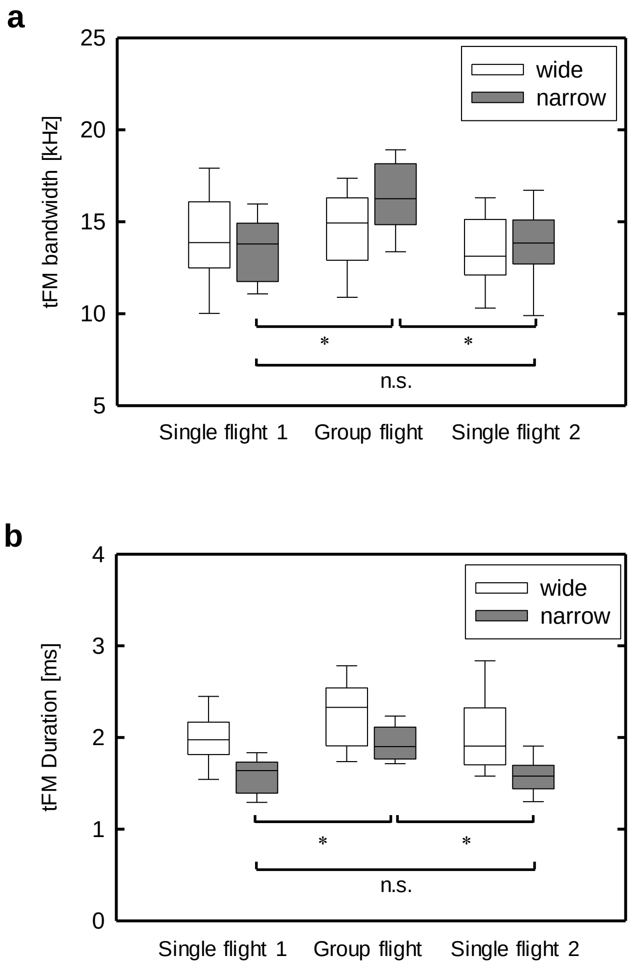
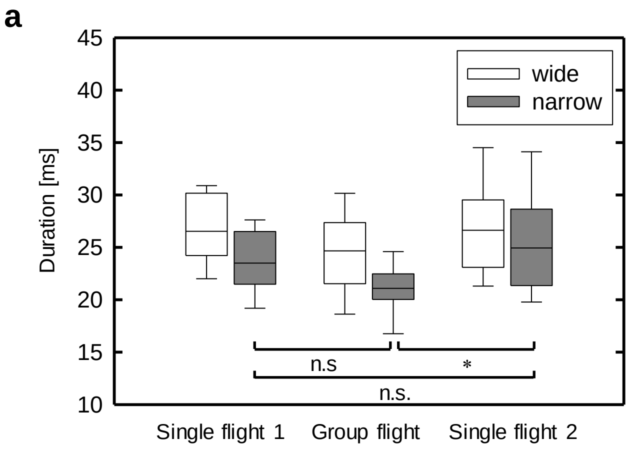


Figure 5-3 Changes in bandwidth (a) and duration (b) of tFM components across flight conditions (single flight 1, group flight, and single flight 2) in the wide and narrow spaces. The horizontal bars above and below the boxes show the 10th and 90th percentiles, respectively.

The duration of CF-FM pulses (pulse duration) was not significantly different among single flight 1 (26.5 ± 3.4 ms), single flight 2 (26.8 ± 5.3 ms), and group flight (24.7 ± 4.0 ms) in the wide space (Fig. 5-4a, one-way ANOVA, $p = 0.317$). Pulse duration was not significantly different from single flight 1 (23.8 ± 2.8 ms) to group flight (21.1 ± 2.7 ms) and single flight 2 (25.3 ± 4.8 ms) but significantly different between group flight and single flight 2 in the narrow space (Fig. 5-4a, Tukey's HSD test, $p < 0.05$).

In addition to the changes in spectro-temporal characteristics of echolocation sounds, we also found changes in temporal patterning of the emissions. Figure 5-4b shows changes in silent time across all flight conditions. The length of silent time was not significantly different among single flight 1 (51.6 ± 6.1 ms), single flight 2 (51.7 ± 9.7 ms), and group flight (54.5 ± 7.1 ms) in the wide chamber (Fig. 5-4 b, one-way ANOVA, $p = 0.453$). Silent time was not significantly different from single flight 1 (50.8 ± 4.0 ms) to group flight (54.3 ± 4.6 ms) and single flight 2 (50.0 ± 3.7 ms) but significantly different between group flight and single flight 2 in the narrow space (Fig. 5-4b, Tukey's HSD test, $p < 0.05$).



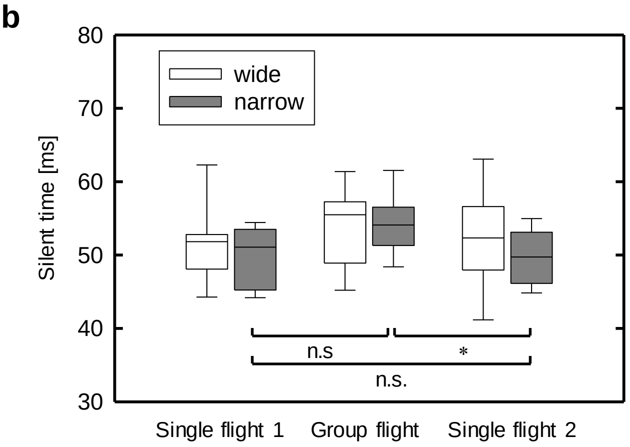


Figure 5-4 Changes in duration (a) and silent time (b) of emitted pulses across flight conditions (single flight 1, group flight, and single flight 2) in the wide and narrow spaces. The horizontal bars above and below the boxes show the 10th and 90th percentiles, respectively.

5. 3. 2. Changes in reference frequency

We investigated how reference frequency was changed in group flight when the bats performed Doppler shift compensation. There was no significant difference in SDs of reference frequency across flight conditions in either wide or narrow spaces (Fig. 5-5a, one-way ANOVA, $p > 0.655$), suggesting that the bats performed Doppler shift compensation in group flight as accurate as in single flight. In the wide space, the SDs of reference frequency were 154 ± 63 Hz in single flight 1, 150 ± 40 Hz in group flight, and

148 ± 47 Hz in single flight 2. In the narrow space, the SDs were 82 ± 22 Hz in single flight 1, 90 ± 23 Hz in group flight, and 85 ± 36 Hz in single flight 2. Data from the wide space were removed from further analysis because the mean SD of the reference frequency was as almost twice large as that in the narrow space, consistent with values from previous studies on the same species (Y. Furusawa et al., 2012). The larger SD could be due to echoes reflected from multiple walls because of a circular flight path in the wide space.

Next, we investigated how reference frequencies differed between flight conditions. We defined ΔRF as the smallest difference in individual reference frequencies. There was no clear pattern in ΔRF (Fig 5-5b). The mean reference frequencies were not significantly different among single flight 1 (68.0 ± 0.4 kHz), single flight 2 (68.0 ± 0.4 kHz), and group flight (68.1 ± 0.4 kHz; Fig. 5-5c, one-way ANOVA, $p = 0.955$).

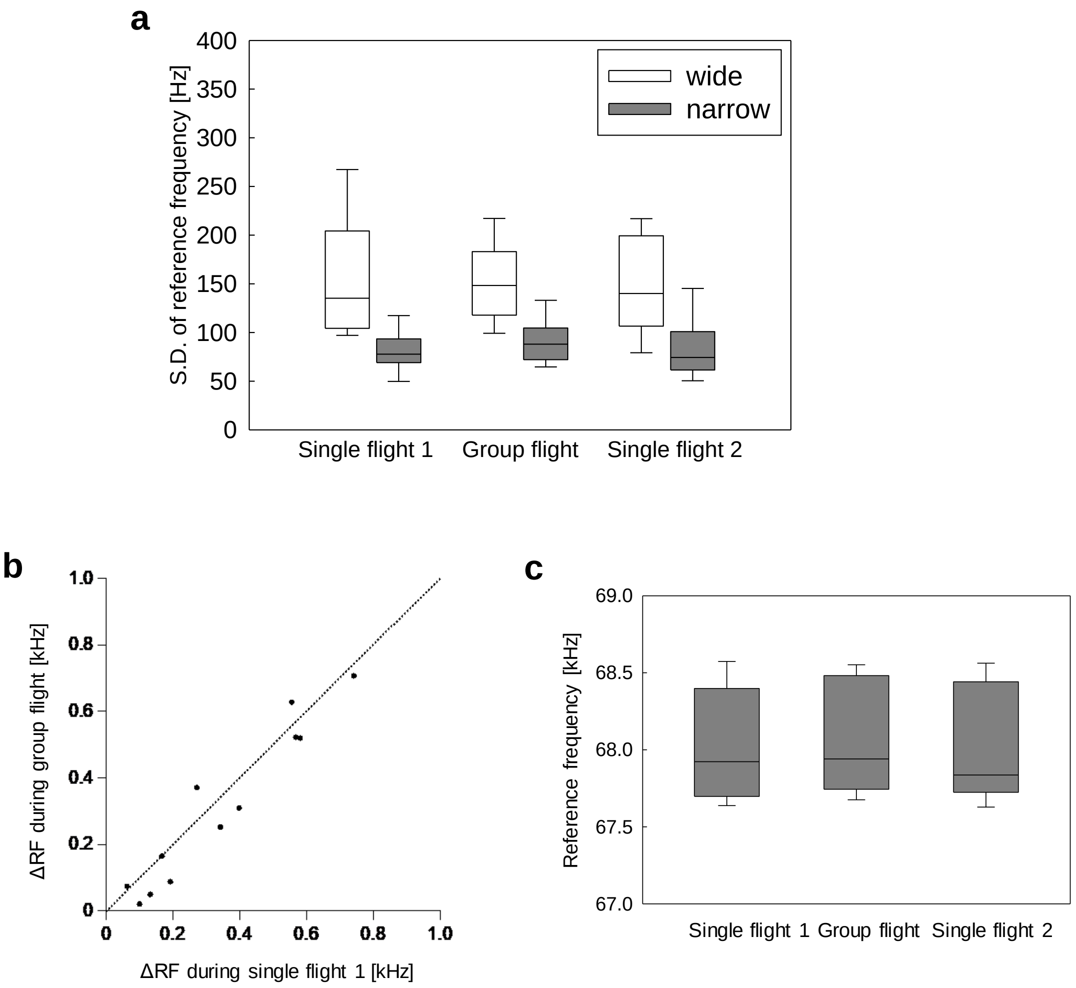


Figure 5-5 (a) Changes in SDs of reference frequency across flight conditions. (b) Relationships between ΔRFs of bats between single and group flights in the narrow space. (c) Changes in reference frequency in the narrow space across flight conditions. The horizontal bars above and below the boxes show the 10th and 90th percentiles, respectively.

5.4. Discussion

In the present study, we recorded echolocation pulses and their returning echoes generated by *R. f. nippon* during group flight and found that the bats decreased pulse duration and increased the duration and bandwidth of the tFM component of the emitted pulses in the narrow space. During group flight, they performed Doppler shift compensation as accurately as in single flight. There were no obvious changes in reference frequency with the presence of conspecifics, suggesting no clear frequency-shifting JARs in CF–FM bats.

Another CF–FM bat species, *R. capensis*, increased the duration and bandwidth of their tFM component during paired flight (K. Fawcett et al., 2015). Similarly, stationary *R. ferrumequinum* broadened the bandwidth of the FM component in their CF–FM pulses in response to band-limited noise (S. R. Hage et al., 2014). *R. ferrumequinum* calculated time differences in FM components between pulses and echoes to measure distances (G. Schuller et al., 1991; J. A. Simmons, 1973; N. Suga & W. E. O'Neill, 1979). Lengthening in duration is thought to improve the signal-to-noise ratio of echoes to corresponding pulse emissions (E. Amichai et al., 2015; P. Heil & H. Neubauer, 2003). These findings suggest that, in the presence of conspecifics, bats improve their range performance by highlighting the tFM components to avoid collision and/or to capture insects.

Furthermore, the bats in the present study decreased their pulse duration and increased their silent time during group flight. *R. capensis* also decreased pulse duration during paired flight (K. Fawcett et al., 2015). Moreover, C. Chiu et al. (2008) demonstrated that when big brown bats flew in pairs, one bat spent a significant amount of time not vocalizing. E. Takahashi et al. (2014) showed that flying Japanese house bats (*Pipistrellus abramus*) emitted more pulses during a silent period than in noise when exposed to band-limited noise bursts. Together, the changes in silent time could decrease temporal overlap of emissions among individuals flying together. Moreover, the present study demonstrated that flying CF–FM bats can modulate their emission timing in the presence of other individuals. Whether the increases in silent time are useful to avoid temporal overlaps between sounds, however, remains a mystery

CF–FM bats have a highly specialized auditory system that is sharply tuned to their reference frequency, called the auditory fovea. Even if the reference frequencies are similar among individuals in a group, the Doppler effect causes adequate frequency shifts for an individual’s auditory fovea to filter vocalizations from other bats. Figure 5-6a shows the representative changes in pulse frequency and returning echoes of one bat in group flight, as well as frequencies of pulses the bat received from the other two bats. The frequencies of pulses the bat received from the other bats dramatically changed across time in comparison with echo frequencies of the bat. Figure 5-6b shows a histogram of the normalized frequencies received by a bat, calculated by subtracting its reference frequency from those of its echoes, and estimates of the other group members' frequencies perceived by the bat. The estimated frequencies of other bats were much more broadly distributed than were the echoes actually received by the bat. The auditory systems of *R. f. nippon* are tuned within fairly narrow ranges corresponding to individual-specific reference frequencies (Fig. 5-7). From these, the auditory fovea may work as a frequency filter to distinguish the bat's own echoes from sounds of other bats.

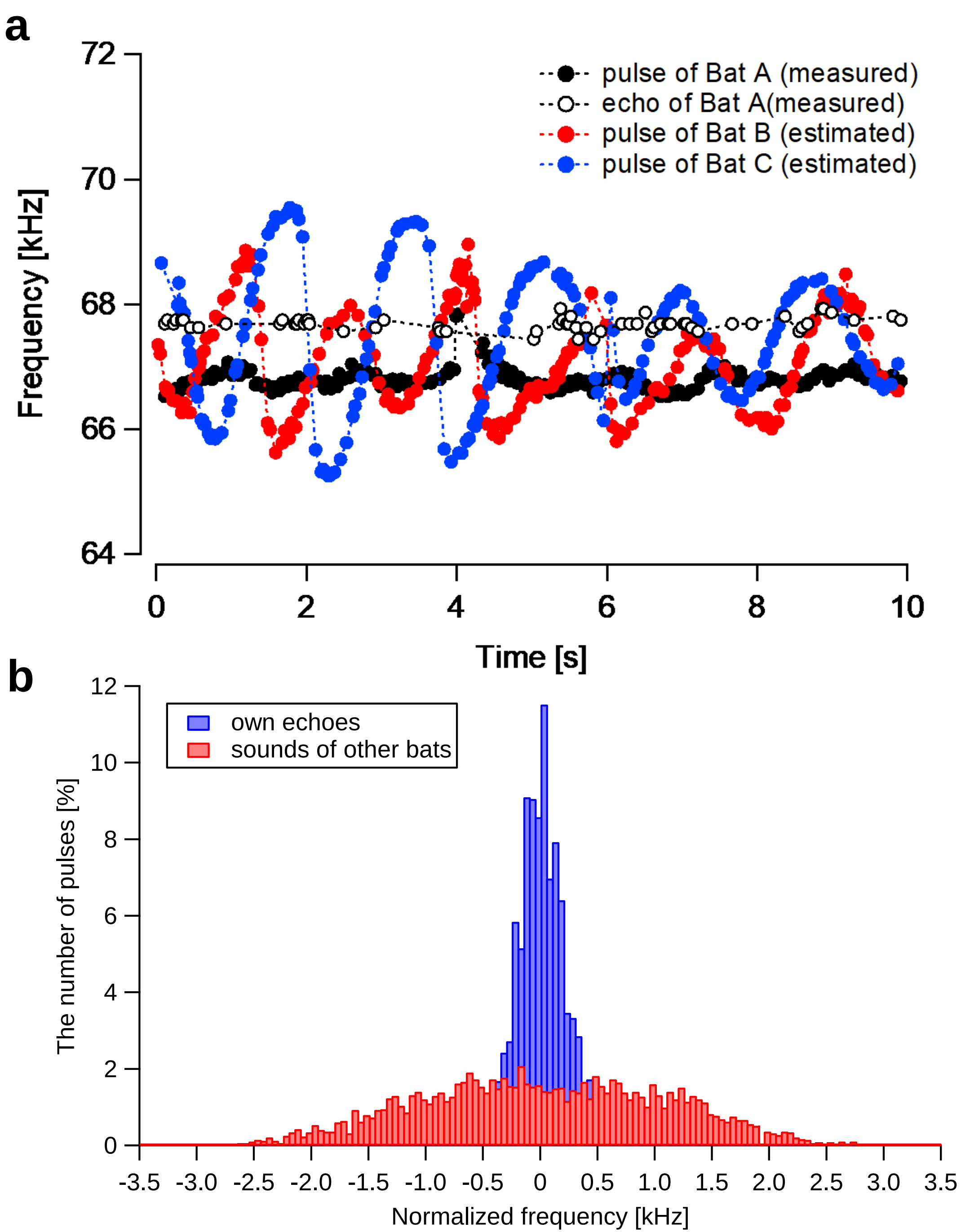


Figure 5-6 Relationships between a bat's reference frequency and the frequencies of pulses it received from other bats. (a) Changes in a bat's echo CF2 frequency (measured) and pulse frequencies it received from the other bats (estimated) during group flight. (b) Distribution of frequency of a bat's own echoes and received pulses of other bats during group flight. The values were normalized by the bat's own reference frequency.

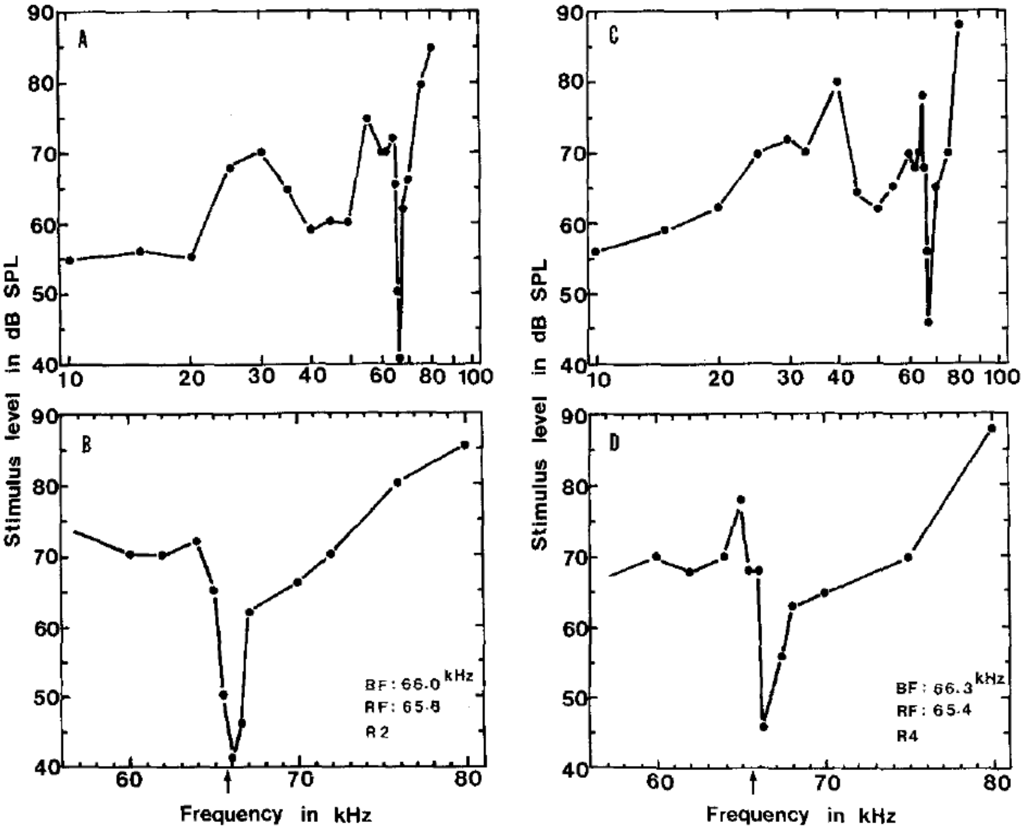


Figure 5-7 Audiograms of two Japanese horseshoe bats measured with a behavioral paradigm (A, C) and enlarged views of the audiograms (B, D). The audiograms have unusual shapes when compared with those of other mammals. Sensitivities are highest at around the reference frequency, and then suddenly decrease at side band frequencies. Figures were adapted from Taniguchi (1985).

Y. Furusawa et al. (2012) demonstrated that the reference frequencies of two Japanese horseshoe bats became more similar when they flew together, whereas we did not observe any clear pattern of changes in reference frequencies across flight conditions. One possible reason for this disparity may be differences in flight tasks. In the study by Y. Furusawa et al. (2012), the bats were trained to land on a front wall in the experimental room, whereas our bats simply flew without a particular task. When two bats fly toward the same stable target, they might conduct Doppler shift compensation to the target. The frequency of echoes of one bat and frequency of pulses received by the bat coming from another bat could be within the range of the auditory fovea of the bat if their reference frequencies were similar. In such a situation, it is still possible that CF–FM bats actively control the reference frequency to solve jamming problems.

5.5. Conclusion

In the present study, we recorded pulses and echoes generated by each individual when three bats were flying together. The bats broadened the bandwidth and lengthened the duration of their tFM component while in group flight, suggesting that they highlight their tFM component to accurately measure distances in the presence of conspecifics. They also decreased pulse duration and increased silent time when flying in groups. This implies that they change their emission timing to avoid temporal overlaps between their echoes and sounds of other bats, although the changes were very slight compared with their silent times. Although the reference frequencies were as precise in group flight as in single flight, there was no clear tendency across flight conditions. By exhibiting Doppler shift compensation behavior, the frequency shifts that occurred with other bat vocalizations due to the Doppler effect may aid the auditory system, which is sharply tuned to individual-specific reference frequencies to extract their weak echoes.

Chapter 6: Dynamics of brainstem auditory evoked potentials in the Japanese house bat (*Pipistrellus abramus*) evaluated with forward masking using frequency-modulated sweeps

The echolocation pulses emitted by bats for orientation and hunting are intense and sometimes exceed 130 dB at 0.1 m from their emitters. In comparison with the emissions, the echoes are relatively weak because of attenuation in the air. When several bats are flying in the same area, a bat may hear the strong pulses emitted by other bats. The masking caused by these strong emissions from other bats can reduce the bat's auditory sensitivity to the echoes of its own emissions. Although studies have reported behavioral adaptations that reduce the masking from other bats, whether and how masking affects hearing sensitivity remains unclear. In this study, we recorded brainstem auditory evoked potentials (BAEPs) from an awake *Pipistrellus abramus* bat during exposure to frequency-modulated sounds mimicking bat echolocation pulses presented alone (echo-only stimulus) or in pair (pulse-echo stimulus). The peak amplitudes of the BAEPs evoked by echo stimuli in the pulse–echo stimuli were lower than those evoked by echo-only stimuli, indicating that auditory forward masking reduced the bat's sensitivity to its own echoes. The masking effects were reduced when the difference in frequency between pulse–echo stimuli was increased by 0.5 kHz. Moreover, when the delay between the pulse–echo stimuli exceeded 20 ms, there were almost no masking effects. Our results suggest that the temporal and spectral jamming avoidance response is useful for reducing the masking caused by the sounds emitted by other bats.

6.1. Introduction

The echolocation pulses emitted by bats are very intense and often exceed 130 dB at 0.1 m from their mouths (L. Jakobsen et al., 2013; A. Surlykke & E. K. Kalko, 2008). Therefore, when several bats are flying together, the intense pulses generated by other individuals can mask the echoes from a bat’s own emissions. In this situation, echolocating bats must process the weak echoes reflected from a small insect to successfully track and capture it. Several mechanisms have been proposed that reduce the masking effects of own strong emissions on their sensitivity to weak echoes. One study found that the peripheral auditory sensitivity evaluated by measuring cochlear microphonic potentials from bats performing echolocation is reduced due to stapedius muscle contraction that occurs before pulse emission (O. Henson Jr, 1965). The muscle contraction reduces the efficiency of wave propagation from the tympanic membrane to the cochlea. P. Jen and N. Suga (1976) demonstrated that contractions of the stapedius and laryngeal muscles are synchronized when bats emit pulses, indicating that corollary discharges contribute to suppressing their sensitivity to intense pulses and then reducing the masking effects.

By contrast, it is still not known how pulses emitted by other bats affect the sensitivity to own echoes, and how bats avoid potential masking by the sounds of other bats. One way to reduce the masking effects of strong sounds from other bats is to simply improve the signal-to-noise ratio (SNR) of echoes by emitting more intense and longer pulses. Several studies have revealed that echolocating bats increase their pulse intensity and lengthen the pulse duration in the presence of various types of noise, which is equivalent to the Lombard effect observed in many other animal species (E. Amichai et al., 2015; S. R. Hage et al., 2013; K. Hase et al., 2016; J. Luo et al., 2017; E. Takahashi et al., 2014; J. Tressler & M. S. Smotherman, 2009). As echolocating bats using downward frequency-modulated (FM) pulses lengthen their duration by increasing the terminal low-frequency portion of the pulses, they can increase the SNR with less negative effects of the attenuation of high-frequency ultrasound in air (B. D. Lawrence & J. A. Simmons, 1982). By increasing the pulse duration, the detectability of echoes also increases because their auditory system can integrate sound over time (P. Heil & H. Neubauer, 2003). However, the Lombard effect *per se* is likely insufficient to reduce the masking effects, as the increase in pulse intensity reported in these studies was only a few decibels.

The masking effects of other bat sounds might be mitigated by another strategy called jamming avoidance responses (JARs). With JARs, signal characteristics are modified to reduce the similarity between own echoes and the sounds of other bats. Studies have shown that echolocating bats change their spectral and temporal characteristics to reduce the similarity when they are confronted with artificial noises (E. Amichai et al., 2015; M. E. Bates et al., 2008; E. H. Gillam & B. K. Montero, 2015; E. H. Gillam et al., 2007; K. Hase et al., 2016; E. Takahashi et al., 2014; J. Tressler & M. S. Smotherman, 2009) or the sounds of conspecifics flying together (C. Chiu et al., 2009; J. Habersetzer, 1981; K. Hase et al., 2018; N. Ulanovsky et al., 2004). Bats appear to facilitate segregation of their own echoes by using JARs. However, it is not clear whether and how JARs reduce masking effects because the inter-individual differences in pulses produced with JARs are not very large.

In this study, we measured the brainstem auditory evoked potentials (BAEPs) in an awake bat in the presence of stimuli mimicking pulse–echo pairs from bats, with various combinations of different echo delays and frequency differences between the pulse and echo. We also recorded BAEPs in the presence of only the echo stimulus used in the pulse–echo stimuli (echo-only stimulus). To quantify the forward-masking effects occurring between pulse-like signals presented in proximity in terms of time and frequency, we investigated the differences in amplitudes of BAEPs evoked by the echo stimuli in pulse–echo stimuli and in echo-only stimuli.

6.2. Materials and Methods

6. 2. 1. Animal preparation

Two male Japanese house bats, *Pipistrellus abramus*, (Bats A and B) were studied. The bats were captured from a colony roosting in bridge girders near the campus of Doshisha University, Japan, under license and in compliance with current Japanese laws. The bats were housed in a cage in a temperature-controlled room and were allowed free access to food and water under a reversed 12-h light/dark cycle. *P. abramus* emit downward FM pulses with several harmonics, and the frequency of the fundamental component is modulated exponentially from approximately 100 to 40 kHz (S. Hiryu et al., 2007).

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

6. 2. 2. Sound stimuli

Pulse and echo stimuli mimicking bat echolocation sounds were generated using MATLAB 2017a. To mimic the time-frequency structure of bat pulses, we used the following formula (S. Parsons & G. Jones, 2000):

$$f(t) = \frac{f_0}{f_0 - af_1}\left\{(f_0 - f_1)\left(\frac{af_1}{f_0}\right)^t + (1 - a)f_1\right\}$$

where f_0 and f_1 indicate the start and end frequencies, respectively, and a is a constant that determines the shape of the sweep. We set a = 0.5, as used in a previous study, to mimic the curvature of the FM sweep of bats (Y. Maitani et al., 2018). We presented pulse–echo stimuli that were a combination of two FM sounds. The pulse stimulus had a minimum frequency of 45 kHz, a maximum frequency of 85 kHz, and a duration of 3 ms. The echo stimuli were frequency-shifted versions of the pulse stimulus, and the shifts were –4, –2, –1, –0.5, –0.2, –0.1, 0, 0.1, 0.2, 0.5, 1, 2, and 4 kHz (ΔF in Fig. 6-1). The delay between the pulse stimulus and each echo stimulus was 5, 10, 20, 40, or 100 ms. We also presented the echo stimuli used in the pulse–echo stimuli alone (echo-only stimuli). The sound pressure levels of the pulse stimuli and echo stimuli at the position of the bat were 80 dB and 60 dB peSPL, respectively. To measure the BAEPs, each stimulus was presented 100 times at 300-ms intervals.

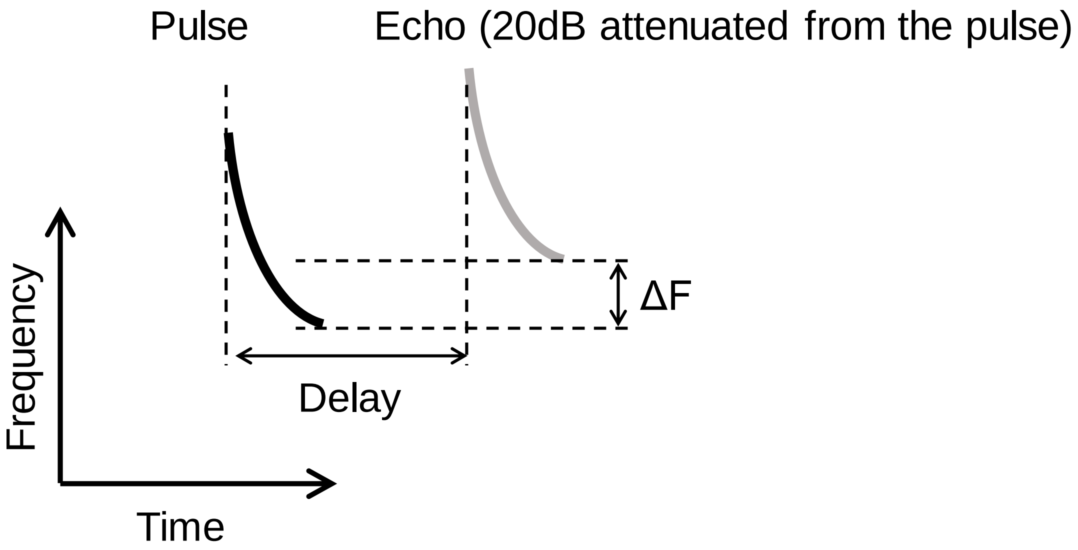


Figure 6-1 Schematic spectrogram of the stimuli used in this study. A pulse stimulus was followed by an echo stimulus at a different frequency from the pulse stimulus (ΔF in the figure), with various echo delays from 5 to 100 ms. We also presented the bats with echo-only stimuli, which were identical to the echo stimuli presented in the pulse–echo stimuli.

6. 2. 3. Recording procedure

For surgery, the bats were anesthetized with inhaled isoflurane. After the skull fur was shaved, a longitudinal midline incision was made through the skin over the skull. The muscle tissue covering the skull was carefully removed. With a fine needle, a tiny hole was made over the inferior colliculus (IC) based on visual inspection, approximately 2 mm posterior and 2 mm lateral from the lambda suture (S. Boku et al., 2015). A metal rod was fixed onto the left anterior part of skull with instant acrylic glue and dental cement. Using the metal rod and custom-made polystyrene foam, we gently fixed the bats during the BAEP recordings. A Teflon-coated silver wire electrode (φ0.127 mm, Nilaco, Tokyo, Japan, impedance ca. 10 kHz) was inserted through the hole and placed on the surface of the right IC as a recording electrode. Another electrode was placed as a reference electrode on the frontal area of the right hemisphere.

The BAEPs were recorded in a sound-proofed, electrically-shielded room using an Intan RHD2000 data-acquisition system with RHD2000 Interface software (Intan Technologies, Los Angeles, CA, USA; Fs = 20 kHz), and stored on a personal computer. The stimuli played through Cool Edit 2000 software (Syntrillium Software Corporation, Phoenix, AZ, USA) were converted using a USB audio interface (UA-101; Roland, Shizuoka, Japan; Fs = 192 kHz), then through a speaker driver (ED1; Tucker Davis Technology, Alachua, FL, USA), and presented from a loudspeaker (ES1; Tucker Davis Technology) located 10 cm in front of the bat's head.

6. 2. 4. Data analysis

We used a digital band-pass filter (300–3 kHz) to reduce the background noise. The recorded waveforms were averaged over 100 repetitions of each stimulus. We focused on the peak amplitudes of the most prominent peak of the evoked BAEPs. To quantify the effects of forward masking, we normalized the amplitudes of the BAEPs evoked by an echo stimulus of the pulse-pair as the peak amplitudes of the BAEPs evoked by the echo-only stimuli at the same frequency. We investigated whether and how the peak BAEP amplitudes evoked by the echo stimuli in pulse–echo stimuli differed from those evoked by echo-only stimuli.

6.3. Results

Figure 6-2 shows representative waveforms of BAEPs recorded from Bat A evoked by each stimulus. When an echo-only stimulus of 85–45 kHz was presented alone, the peak value of the BAEP was 49.6 µV (Fig. 6-2a). In the presence of the pulse–echo stimuli with a 5-ms echo delay and 0-kHz frequency difference, the peak amplitude of the BAEP evoked by the echo stimulus of the pair was 30.7 µV, showing a 5.6 dB reduction from that evoked by the echo-only stimulus (Fig. 6-2b). In comparison, when exposed to the pulse–echo stimuli with a 40-ms echo delay and 0-kHz frequency difference, the peak amplitude evoked by the echo stimulus of the pair was 47.7 µV, which was almost the same as that evoked by the echo-only stimulus (Fig. 6-2c). In contrast, the peak values of the BAEPs evoked by the pulse stimuli of these two pulse–echo stimuli did not show a clear reduction or increase, and were 64.7 µV and 66.2 µV, respectively.

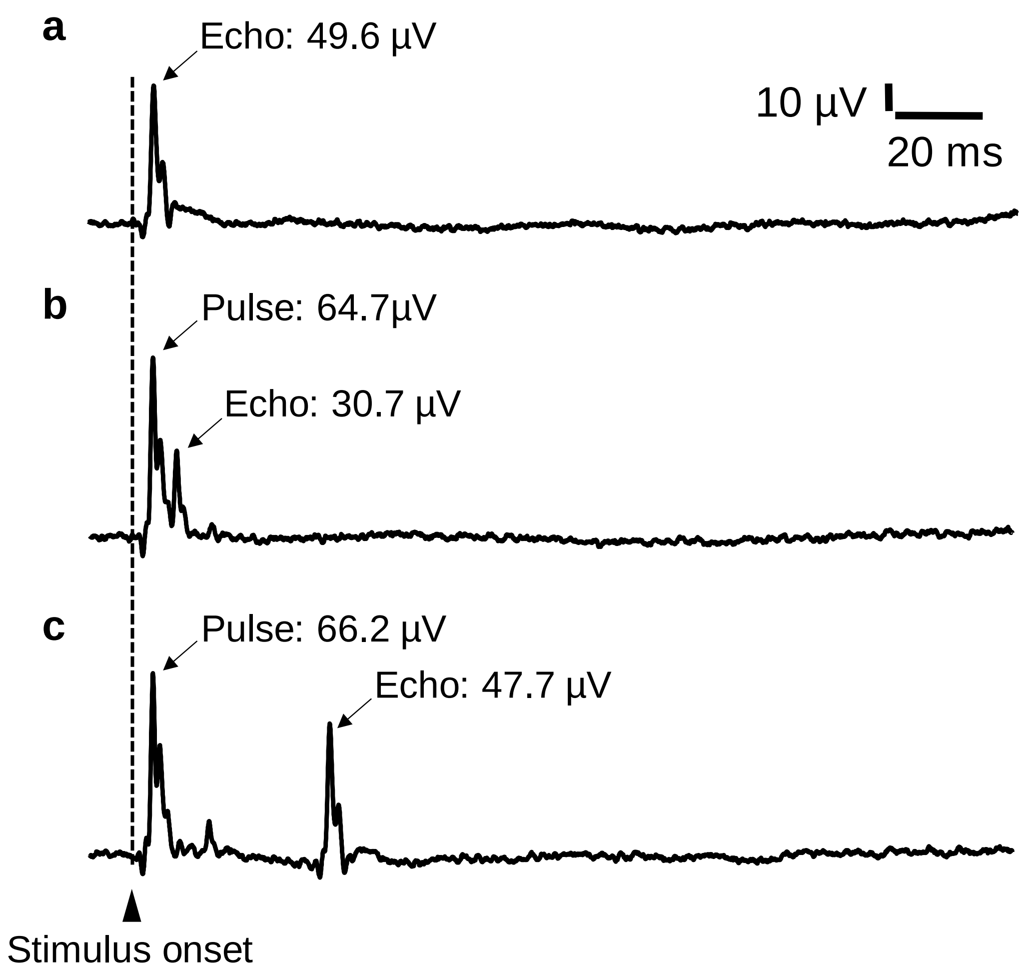


Figure 6-2 Representative waveforms of the BAEPs recoded from Bat A evoked by an echo-only stimulus of 85–45 kHz (a), a pulse-echo stimulus when the pulse stimulus had the same frequency as the echo stimulus with an echo delay of 5 ms (b), and a pulse-echo stimulus with an echo delay of 40 ms (c). The peak amplitude evoked by the echo-only stimulus was 49.6 µV, whereas that evoked by the echo stimulus of the pulse–echo stimulus with an echo delay of 5 ms was reduced to 30.7 µV. The peak amplitude evoked by the echo stimulus of the pulse–echo stimulus with an echo delay of 40 ms showed moderate recovery.

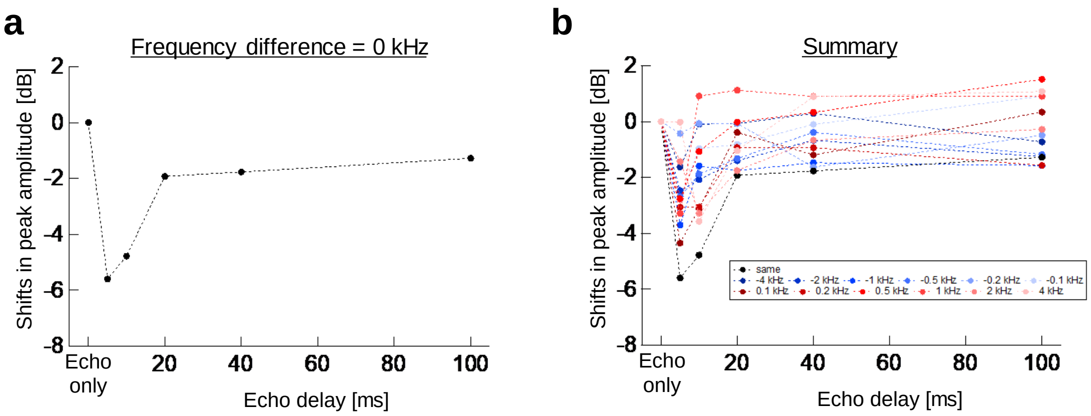


Figure 6-3 Time course of peak amplitudes of BAEPs evoked by echo stimuli when frequency differences in pulse and echo stimuli were 0 kHz (a) and Summary of the results.

First, we investigated the temporal changes in the BAEPs. Figure 6-3 shows the changes in the masking effect. Here, the masking effects were quantified by normalizing the peak amplitudes of BAEPs evoked by the echo stimuli in the pulse–echo stimuli by the peak amplitudes of the echo-only stimuli for each echo frequency. When an echo stimulus of 85–45 kHz was presented 5-ms after a pulse stimulus of the same frequency, the masking effect was 5.6 dB (Fig. 6-3 a). When the echo frequency was shifted from the pulse stimulus, the masking effect was reduced, but we observed the maximum effect of masking with an echo delay of 5 ms (Fig. 6-3b).

Next, we focused on how the masking effects changed with the frequency differences in the pulse–echo stimuli. Figure 6-4 shows the relationships between the masking effects and the frequency difference in the stimuli. When the difference was from –0.2 to 0.2 kHz, larger effects were observed. When the difference was increased to –0.5 or 0.5 kHz, we observed a moderate reduction in the masking effect. This trend was observed for all echo delays of the pulse–echo stimuli.

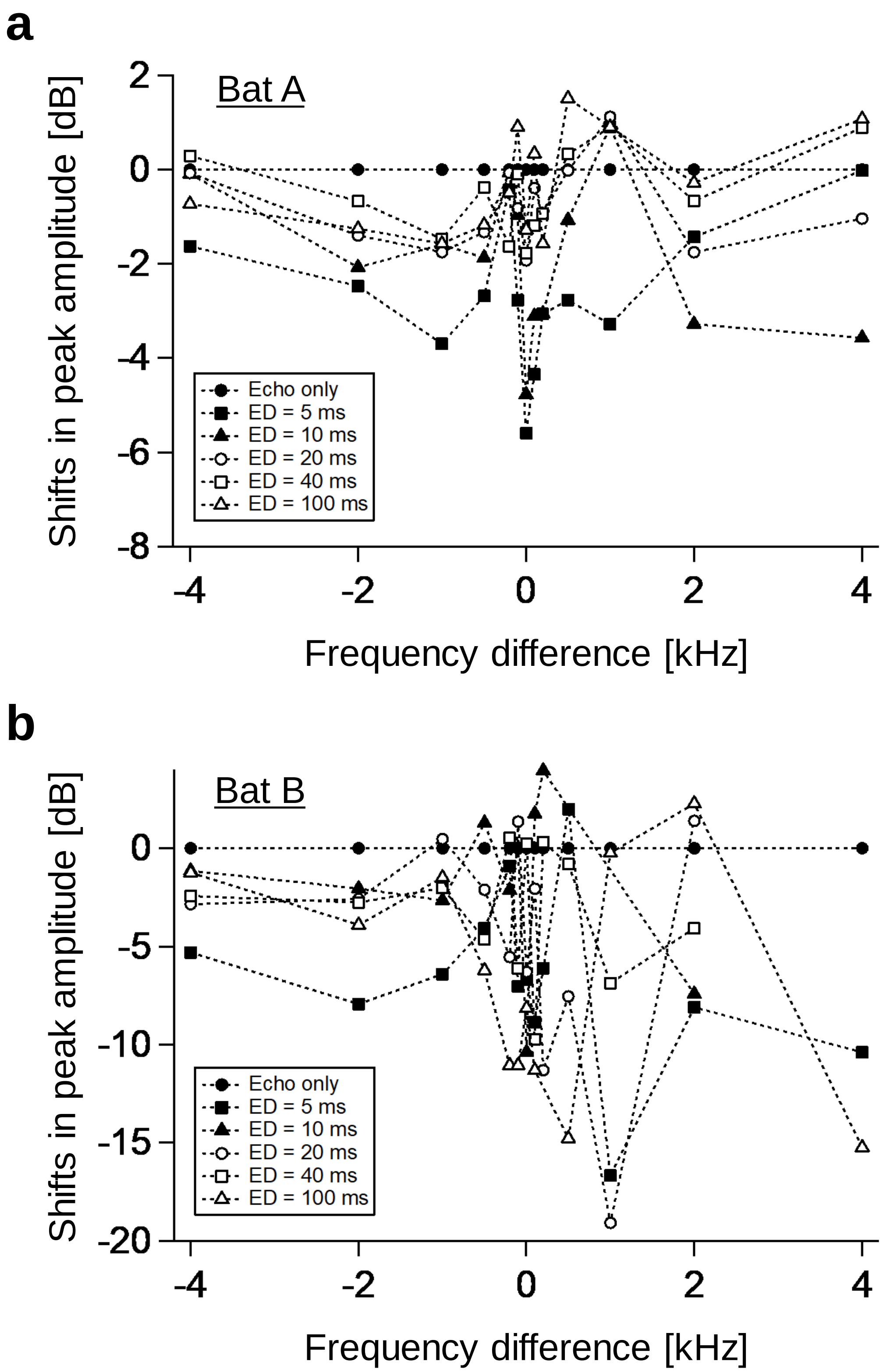


Figure 6-4 Shifts in the peak amplitudes of BAEPs from Bat A (a) and Bat B (b) evoked by the echoes in the pulse–echo stimuli normalized to the echo-only stimulus in response to the frequency shifts between the pulse and echo stimuli.

6.4. Discussion

In this study, we characterized the effects of forward masking occurring in two successively presented bat-like acoustic signals that differed in delay and frequency by measuring BAEPs in an awake bat. We observed slight decreases in the peak amplitudes of BAEPs evoked by echo stimuli when the two signals were presented at shorter time differences (delays of 5 or 10 ms). These masking effects were reduced when there were frequency differences of at least 0.5 kHz.

The problem of auditory masking due to strong own emissions is solved by reduced peripheral sensitivity immediately before pulse emission through contraction of the middle ear muscles synchronized with laryngeal muscle activation (O. Henson Jr, 1965; P. Jen & N. Suga, 1976). Bats can use this mechanism to become less sensitive to their own strong emissions, as they know when they emit pulses. In contrast, because the timing of strong pulses coming from other individuals is much more unexpected, they need another mechanism to reduce forward or backward masking from the sounds of other bats. Our data show that the effects of masking were reduced when pulse–echo stimuli had frequency differences as small as 0.5 kHz, suggesting that either cochlear mechanics or the regulation of auditory peripheral sensitivity via projection from higher auditory centers is involved in the release from the masking, although the results were not sufficient to explain the mechanism.

Several studies have indicated that echolocating bats use frequency-shifting JARs to avoid or mitigate jamming. N. Ulanovsky et al. (2004) demonstrated that *Tadarida teniotis* flying in pairs separated the terminal frequency of their emitted pulses. In the presence of constant-frequency tones, *Eptesicus fuscus* engaging in target detection tasks shifted their terminal frequency away from that of the presented tone (M. E. Bates et al., 2008). The shifts in frequency of JARs observed in previous studies were only a few kHz. In contrast, the bandwidths of their emitted pulses were relatively broad, and ranged from 20 to 100 kHz, including prominent harmonic components. Do echolocating bats recognize the slight differences in spectral characteristics between pulses, which correspond to at most 10% of the entire bandwidth of the pulses?

Using a telemetry recording technique that allowed the authors to capture sounds emitted by all group members separately, K. Hase et al. (2018) demonstrated that *Miniopterus fuliginosus* flying in a group of four bats shifted the terminal frequency of their emitted pulses away from each other. They showed that the similarity of pulses among individuals decreased during group flight when compared with single flight, and suggested that shifts in terminal frequency are more effective for segregating bat-like signals than shifts in other acoustic parameters. It was also suggested that echoes coming from off-axis objects could be "defocused" by comparing spectral features in the emitted pulses and echoes because their emitted pulses are highly directional, and higher frequencies are more directional (M. E. Bates et al., 2011). These studies suggest that bats may recognize slight spectral differences in sounds to reduce jamming or masking from sounds of other individuals and behaviorally irrelevant echoes from their own signals.

Moreover, the effects of forward masking may be reduced by increasing the time difference between the maskee (i.e., own weak echoes) and masker (i.e., strong emissions of other bats or even themselves). Stationary *Tadarida brasiliensis* suppress their emission rate tens of milliseconds after the presentation of noise (J. Jarvis et al., 2010). Some studies reported that echolocating bats stop emitting pulses when they are flying with conspecifics (A. M. Adams et al., 2017; C. Chiu et al., 2008). Bats may stop emitting pulses or reduce the number of pulses emitted as a sound group per wingbeat cycle, because it has been demonstrated that the timing of emission and wingbeat are strongly correlated during flight (B. Falk et al., 2015). E. Takahashi et al. (2014) showed that *P. abramus* emit more pulses in the presence of band-limited noise bursts than in silent periods, suggesting that flying bats adaptively modulate their emission timing to avoid masking caused by noise. Therefore, it is possible that echolocating bats postpone their pulse emissions to avoid forward masking from pulses emitted by other bats. Although the problem of masking and jamming by other bats seems severe, by combining several mechanisms, bats can facilitate the extraction of weak echoes in the presence of noisy conspecifics.

Chapter 7: General Discussion

7.1. Summary of Results

In this dissertation, I conducted a series of experiments to elucidate how echolocating bats extract their own weak echoes in the presence of jamming signals. Here, I briefly summarize the results.

7. 1. 1. Rapid, adaptive changes in pulse characteristics in response to artificial jamming sounds (Chapters 2 and 3)

In Chapters 2 and 3, I presented various jamming sounds to *Miniopterus fuliginosus*. I used a telemetry microphone to capture emitted pulses during flight, which allowed me to measure the acoustic characteristics of the pulses accurately, without any distortion caused by attenuation in the air or the Doppler effect. I found that echolocating bats rapidly (within 150 ms) shifted the terminal frequency (TF) of the emitted downward frequency-modulated (FM) pulses in response to the jamming sounds. I also found adaptive changes in TF depending on the TF of the presented jamming signals that mimicked bat echolocation pulses. The bats shifted their TF upward in response to the pulse mimics with a TF lower than those of their own pulses but did not significantly change TF in the presence of pulse mimics with a higher TF. Additionally, such upward shifts in TF were elicited not only by pulse mimics (exponentially modulated downward FM sounds) but also by a time-reversed version of the pulse mimics, constant-frequency (CF) tone bursts. In comparison, downward and upward linear FM sounds did not induce upward shifts. These results suggest that echolocating bats can adaptively change the acoustic characteristics of their emitted pulses depending on the immediate changes in the auditory information they receive. The results also imply that the adaptive changes are triggered by the spectral information contained in the jamming sounds, rather than by spectro-temporal information.

7. 1. 2. Echolocation behavior of group-flying FM bats revealed with a telemetry microphone system (Chapter 4)

After converting the telemetry microphone system into a multi-channel recording system, I recorded the sounds of each bat flying in a group. I found that the group-flying bats shifted the TFs of their FM pulses away from each other to broaden the inter-individual differences in TF during single flight. The similarities in their pulses decreased significantly in group flight compared with in single flight. I also found that this strategy is useful for segregating echolocation sounds because the similarity among FM signals mimicking the bat pulse was decreased maximally with minimal TF manipulation. Our results show that universal rules applicable to animals using active sensing, such as echolocating bats and electric fish, transcend species and sensory modality borders.

7. 1. 3. Doppler shift compensation contributes to reducing spectral jamming in CF-FM bats during group flight (Chapter 5)

In Chapter 5, I described an experiment similar to that addressed in Chapter 4 but used a different species of bats, *Rhinolophus ferrumequinum nippon*, which emits CF-FM echolocation pulses. *R. f. nippon* is known to compensate its echo frequency within a very restricted range, called the reference frequency, by controlling the frequency of emitted pulses to cancel the frequency changes in the echoes induced by the Doppler effect caused by their flight (Doppler shift compensation). Using the multi-channel telemetry microphone system, I measured not only pulses but also echoes from each individual flying in a group of three bats. During group flight, the bats increased the bandwidth and duration of the terminal FM components of the emitted pulses compared with during single flight, suggesting that the changes resulted in improved range discriminability due to the rich spectral information obtained from echoes.

However, the frequency of the returning echoes did not change significantly from single flight to group flight. I found that the frequency of the received pulses emitted by the other bats varied much more, whereas their echo frequency was maintained within a fairly narrow range by Doppler shift compensation. This suggests that echolocating CF-FM bats could reduce spectral jamming from other bats by using narrow auditory filters tuned to their individual-specific reference frequencies.

7. 1. 4. Effects of strong sounds emitted by other bats on sensitivity to one’s own echoes

(Chapter 6)

Finally, I examined whether and how auditory masking from strong pulse emission affects the bats' perceptions of their own faint echoes. I recorded brainstem auditory evoked potentials (BAEPs) from an awake *Pipistrellus abramus* while the bat was exposed to the single (echo-only stimuli) or double (pulse-echo stimuli) FM sounds mimicking bat echolocation pulses. The peak amplitudes of the BAEPs evoked by echo stimuli in the pulse-echo stimuli were decreased compared with those evoked by echo-only stimuli, indicating that auditory forward masking from the sounds of other bats reduced the bats' sensitivity to their own echoes. The masking effects were reduced when the difference in frequency between pulse-echo stimuli increased by 0.5 kHz. Additionally, when the delay between the pulse-echo stimuli exceeded 20 ms, there were almost no masking effects. These results suggest that the frequency-shifting jamming avoidance response (JAR) is useful for reducing the forward masking from sounds of other bats.

7.2. How does each FM bat flying in a group determine the emission frequency?

Echolocating bats receive various sensory inputs, including insect echoes, clutter echoes, and pulses and echoes from other bats. This must create a complex acoustic situation. It seems difficult for a bat to find an "open slot" and adapt its pulse frequency to the changing acoustic situation. There is evidence that echolocating bats can adaptively change the frequency of their emitted pulses immediately after they receive one jamming sound (J. Luo & C. F. Moss, 2017). This suggests that a complex acoustic situation that changes immediately with the activity of surrounding bats shapes the echolocation behavior of a given bat.

The results from Chapter 4 suggest that the direction of frequency shifts during group flight depends on the relationships among the frequencies of the pulses they emitted during single flights. In other words, the inter-individual differences in the frequencies of emitted pulses during single flight were broadened in group flight. This is convincing because broadening their original inter-individual differences requires a smaller change in frequency by each animal. Nevertheless, because bats are social animals, it is still possible that frequency allocation during JARs is based on their hierarchy. Bats possess a rich repertoire of communication signals (M. A. Gadziola et al., 2012; J. Ma et al., 2006). The communication signals may be used among bats flying in groups to determine in which direction they change frequencies. Although only one communication call was observed in the study examined in Chapter 4, this possibility needs to be investigated both in species that often use communication calls in flight and in species that rarely rely on communication calls in flight.

7.3. How fast can a bat adaptively change pulse characteristics in response to jamming?

In this study, we showed that *Miniopterus fuliginosus* changed TF within 150 ms in response to a jamming stimulus. Some previous research suggested that FM bats shift their frequency of emitted sounds 150~200 ms after the presentation of jamming sounds (E. H. Gillam & B. K. Montero, 2015; E. H. Gillam et al., 2007). J. Luo and C. F. Moss (2017) demonstrated that *Eptesicus fuscus* adaptively shifts the TF of pulses with a latency of 60~90 ms when the jamming stimuli mimicked echolocation pulses. Similarly, in the presence of band-limited noise, the amplitudes and frequency of pulses were increased in the very first pulses emitted after presentation (S. R. Hage et al., 2013). J. Luo et al. (2017) reported that the response latency of the Lombard effect of bats was only 30 ms when they . This research suggests that echolocating bats can make adaptive changes in pulse characteristics depending on the immediate sensory input. Further investigation is needed to confirm how fast they can respond to the immediate input. If a bat successively receives two or more sounds with different characteristics, how does the bat respond?

7.4. What is the possible neural circuit controlling the JAR?

I demonstrated that JAR was elicited by not only jamming stimuli mimicking pulses but also by time-reversed pulse mimics. E. Amichai et al. (2015) also reported that *Pipistrellus kuhlii* shifts its frequency in response to both conspecific sounds and time-reversed versions. These results imply that spectral information is important for avoiding jamming. By contrast, the ranging performance of *Eptesicus fuscus* was degraded when a time-reversed pulse was presented as the echo of their emission, suggesting that spectro-temporal information is critical for analyzing echoes (W. M. Masters & S. C. Jacobs, 1989). If they can discriminate normal and time-reversed pulses, why do they shift frequency?

This can be explained in several ways. First, by shifting the frequency away from jamming stimuli, they can reduce spectral masking. In FM echolocation sounds, prominent spectral peaks are observed around their TF. Moreover, listening to the lower frequency portion of echoes is critical for measuring target distances accurately (M. E. Bates & J. A. Simmons, 2010). Therefore, by reducing the spectral masking at the level of the basilar membrane, they can improve the signal-to-noise ratio of echoes, especially in the frequency ranges around their TF.

Next, the frequency shifts result in sound segregation. Bats change the acoustic characteristics of emitted pulses immediately after jamming sounds are presented(J. Luo & C. F. Moss, 2017). The rapid latency of responses is comparable to the response latency of the Lombard effect, which has essential circuits in the brainstem (S. R. Hage et al., 2006), suggesting that JAR also involves neural circuits at a brainstem level. Frequency shifts performed by lower levels of the auditory system could facilitate echo extraction from the sounds of other bats in a higher auditory center.

7.5. Possible mechanism for extracting one's own echoes from sounds of other bats?

M. E. Bates et al. (2011) suggested that clutter echoes coming from off-axis objects are defocused by desynchronization of the neural responses in each frequency contained in an FM sound due to amplitude–latency trading given that the echoes are low-pass filtered by their directional beam pattern. When two bats flying together use the same TF, there should obviously be some differences in spectro-temporal structure. Bats might use these differences to "defocus" the sounds of other bats and extract their own echoes. The problems of clutter and jamming by conspecifics may be solved by the same mechanism.

Echolocation might also involve higher-level brain functions. Attention could modulate their auditory sensitivity to focus on a specific frequency channel and a specific echo delay. These attentional effects can facilitate echo extraction in a complex acoustic situation caused by multiple bats flying together. To elucidate how attention works in bat echolocation, further study should combine behavioral experiments and neurophysiological recordings, pharmacological techniques, or even optogenetics.

7.6. Active motor behavior could reduce jamming

A motor behavior other than controlling the characteristics of vocalization could be effective in reducing jamming. Echolocating bats can broaden their directional beam actively before capturing insect prey so as not to lose it from their acoustic field of view (L. Jakobsen & A. Surlykke, 2010; N. Matsuta et al., 2013). Echolocating bats also change the direction of their sonar beam rapidly by 90 degrees within successive pulses (E. Fujioka et al., 2014). Additionally, M. J. Wohlgemuth et al. (2016a) demonstrated that *Eptesicus fuscus* increased pinna separation during echo reception to maximize interaural acoustic cues. Therefore, bats can actively control the directionality of emitters and receivers to reduce jamming from other individuals when they fly in groups.

Interestingly, it has been reported that echolocation bats optimize their fight paths so that they can catch two insects within 1 second (E. Fujioka et al., 2016). By changing flight direction and velocity, they may control the intensity and frequency of pulses coming from other bats. The jamming avoidance from the perspective of their movements should be assessed.

7.7. Future directions

Technological advances enable us to access the neural activity of freely behaving animals. Although echolocating bats are very small, some studies have made neural recordings from freely moving bats (N. Ulanovsky & C. F. Moss, 2007, 2011). Recently, N. B. Kothari et al. (2018) recorded the echolocation sounds and neural activity of freely flying *Eptesicus fuscus* in a room that contained obstacles, revealing that a population of neurons encodes the position of an object in three-dimensional space. The recording of neural activity, echolocation pulses, and echoes from freely flying bats provide a new opportunity to study how they process their own echoes in the presence of conspecifics.

Studies of bat echolocation in noisy situations could also solve technological problems in artificial auto-communication systems, such as radar or sonar. L. Carrer and L. Bruzzone (2017) suggest that the clutter rejection mechanism of *E. fuscus* can be applied to the radar geophysical exploration of planetary bodies. In this dissertation, we observed some of the adaptations made by echolocating bats to noisy conspecifics, which could have future engineering applications.

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2. **Hase, K.**, Miyamoto, T., Kobayasi, K. I., & Hiryu, S. (2016). Rapid frequency control of sonar sounds by the FM bat, *Miniopterus fuliginosus*, in response to spectral overlap. *Behavioural processes*, *128*, 126-133.
3. Ito, T., Furuyama, T., **Hase, K.**, Kobayasi, K. I., & Hiryu, S. (2018). Organization of projection from brainstem auditory nuclei to the inferior colliculus of Japanese house bat (*Pipistrellus abramus*). *Brain and behavior*, *8*(8), e01059.
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5. Tetsufumi Ito, Takafumi Furuyama, **Kazuma Hase**, Kohta I. Kobayasi, Shizuko Hiryu and Riquimaroux, H. Organization of subcortical auditory nuclei of Japanese house bat (*Pipistrellus abramus*) identified with cytoarchitecture and molecular expression. *Journal of Comparative Neurology* (in press).
6. Furuyama, T., **Hase, K.**, Kobayasi, K. I., & Hiryu, S. Hearing sensitivity evaluated

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Professional memberships

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