# Perceptual Challenges in Bat Echolocation:

# Jamming Avoidance and Clutter Rejection

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## Table of Contents

## List of Tables

## List of Figures

# GENERAL INTRODUCTION

Echolocation, or biosonar, is an active sensory system in which emitted acoustic signals are compared to echoes returning from objects in the environment. It is most sophisticated in bats and toothed whales, which use it to orient and navigate when vision is ineffective (e.g., at night or in murky water). Although echolocation is used primarily for orientation, bats also use it to detect, localize and identify their prey. Echolocating bats are able to form an acoustic image of their world derived from echoes of their emitted sounds.

The echolocation calls of bats vary considerably between species in such acoustic parameters as bandwidth, intensity, duration, and duty cycle (reviewed in Grinnell, 1995). Many bats also possess the behavioral flexibility necessary to adapt features of their calls to suit the needs of different environments. Bats can be divided very generally into two types according to the predominant features of their echolocation calls. Some species primarily use relatively short, frequency-modulated (FM) sounds that sweep through about an octave and contain one or more harmonics that increase their bandwidth. Signals from these bats typically last several milliseconds and are emitted 2-10 times per second while searching for prey. When the bat detects a target of interest, it increases the repetition rate and decreases the duration of the signals, culminating in a terminal buzz, with pulses emitted as rapidly as 100-200 per second (Fig. 1). FM signals are well-adapted to the localization of targets. There seems to be a correlation between bat species that regularly operate in dense vegetation and the use of wideband FM signals containing multiple harmonics (Schnitzler et al., 2003; Simmons et al., 1979). Other species of bats

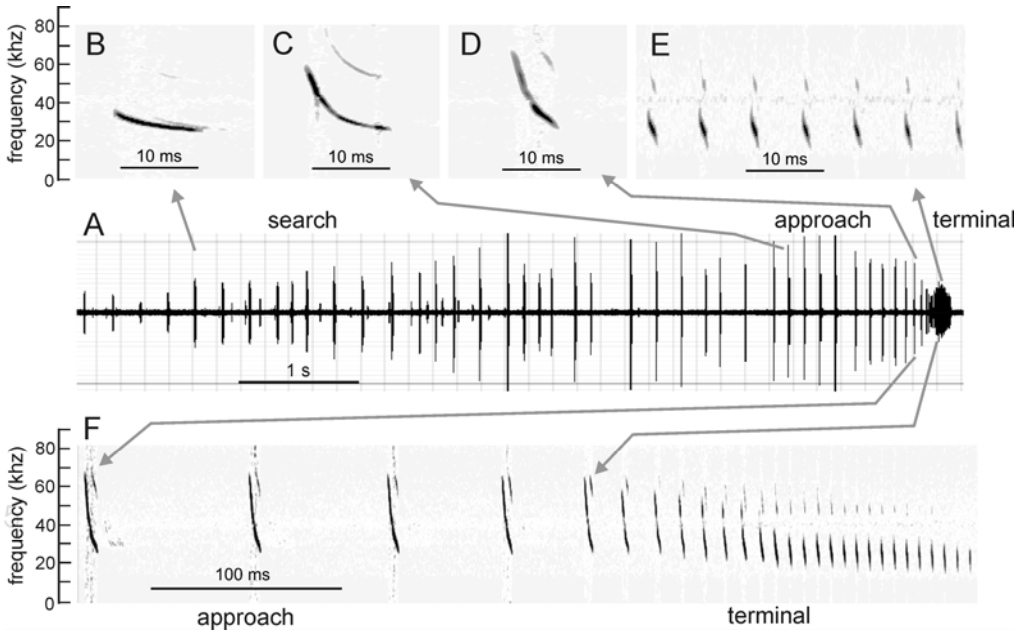


**Fig. 1.** Representative echolocation sequence recorded from a free-flying big brown bat. (*A*) Entire sequence with three phases labeled. During search phase, the bat emits FM sounds with shallow sweeps at regular intervals. When it detects a target of interest, such as an insect, it shifts into the approach phase, emitting steeper, shorter FM sweeps at a faster rate. The terminal phase occurs immediately before capture and is characterized by a significant decrease in interpulse interval. (*B*) Close-up spectrogram (x-axis: time, y-axis: frequency) of single sweep from search phase. (*C,D*) Close-up spectrograms of sweeps from early and late approach phase. (*E*) Close-up spectrogram showing several emissions from the mid-terminal phase. (*F*) Spectrogram showing train of sounds from approach to terminal phase. In *B-F*, the first harmonic (FM1) is the strongest, but higher harmonics (i.e., FM2, FM3) are visible in some of the spectrograms.

emit primarily narrowband signals that are relatively long in duration (up to 100 ms). These constant-frequency (CF) signals are specialized for target detection, especially at long ranges. A large portion of the inner ear and most of the neurons in all the auditory centers of CF bats are sharply tuned to the CF frequency, a neural configuration termed an acoustic fovea. CF signals are used to detect fluttering insects against the clutter of background vegetation. CF bats compensate for Doppler shifts induced by their own flight speed by lowering the frequency of their emitted calls so that the returning echo

falls within the frequency range of the acoustic fovea, where their sensitivity is greatest (Neuweiler, 2000).

A wealth of information is contained within the echoes returning to bats from their environment. Echolocating bats can not only extract from echoes time-domain (range) information, but also frequency-domain or spectral (shape) information. FM bats can perceive the size of a target from the intensity of the echoes (Simmons and Vernon, 1971), the shape of an object from the echo spectrum (Simmons et al., 1974) and the distance to a target from the time delay of echoes after emitted sounds (Simmons, 1973). CF bats use the Doppler shift of echoes to perceive the velocity of a target (Schnitzler, 1968). Information about object structure is contained both in the time and in the spectral interference patterns of signals reflected from surfaces at different distances from the bat (Schmidt, 1988). FM bats have been shown to discriminate among targets that reflect echoes differing in spectral distribution of energy but not in overall intensity, and to detect differences smaller than 1 mm in fine target structure (Simmons et al., 1974). These abilities may enable bats to classify targets based on their echo spectral signatures. Computer-generated FM sonar pulses broadcast at different targets showed specific echo signatures for the different insect species ensonified, which changed with the angle of incident sound (Moss and Zageski, 1994). It is possible that bats also distinguish among flying insect prey based on the echo spectrum.

Echolocating bats depend upon their sense of hearing to navigate among obstacles, position themselves in relation to their environment, and to detect, localize and identify potential prey items. The importance of this sensory modality is evident in the degree to which most bats are able to successfully perform these tasks in the absence of

other sensory input. Two of the most computationally sophisticated, and essential, behaviors that bats must perform are clutter rejection and jamming avoidance (Fig. 2). While flying, bats must be able to detect targets and obstacles in their path while also rejecting echoes from sources of clutter, such as dense vegetation and background objects. Distinguishing the echoes of targets from the echoes of nearby clutter is a challenging task, especially when it is not uncommon for clutter echoes to be more intense than those from small targets and when both types of echoes can be located within the same time window. Jamming, or masking, from the sound-echo pairs emitted by other bats is a potential problem for bats due to their social nature and propensity to live and forage in close proximity to one another. Bats must be able to avoid jamming from the emissions of conspecifics and also be able to distinguish their own echoes from the echoes of other bats' sounds.

In this dissertation, I discuss these two processes - clutter rejection and jamming avoidance - as perceptual challenges for echolocating bats. They are related in that they both require the bat to match the returning echo of its sound to a stored internal representation, or template, of its emission. My experiments focus on a single FM species, the big brown bat (*Eptesicus fuscus*). Big brown bats emit wideband, downward-sweeping FM echolocation sounds (Fig. 1). Although they are commonly thought of as aerial hawking bats, catching insects on the wing, they also regularly feed on insects such as crickets and katydids that are not capable of flight, and so must be taken off of substrates. Thermal video cameras and ultrasonic microphones have recorded big brown bats capturing insects near or possibly on the ground and near or in vegetation (Moss et al., 2006; Simmons, 2005; Simmons et al., 2001). Big brown bats are highly social and

often forage in close proximity to one another. Videos of several bats hunting together, and even bats chasing each other, show that they have no problem orienting and capturing prey with other bats in close proximity (Moss et al., 2006; Simmons, 2005; Simmons et al., 2001).

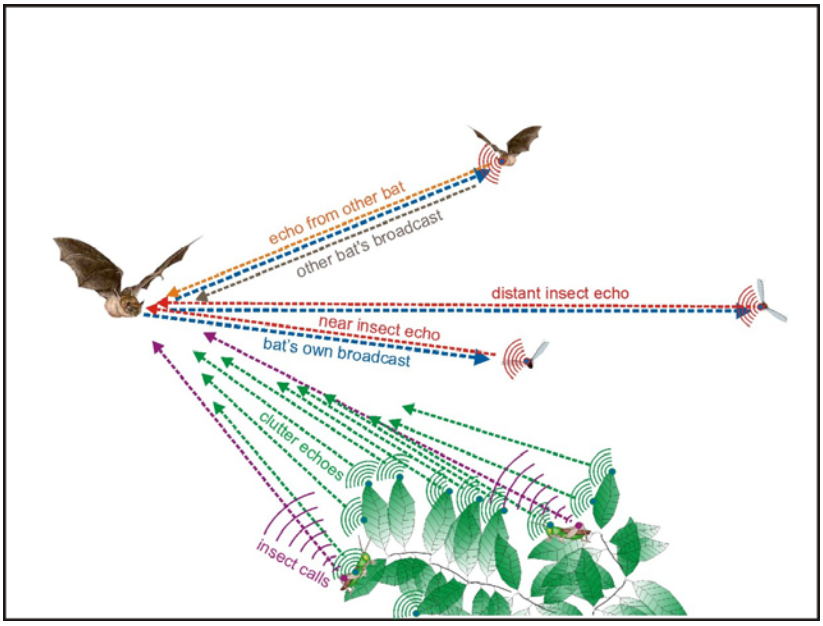


**Fig. 2.** Illustration of perceptual difficulties faced by echolocating bats. A bat emits a biosonar broadcast (blue arrow) and the sound is reflected off of objects in its environment – in this case, two insects at different distances (red arrows), a conspecific foraging nearby (orange arrow), and vegetation in the background (green arrows) are all returning echoes to the bat. Other sounds are also heard by the bat, such as the biosonar broadcasts of the conspecific (gray arrow) and the calls of nocturnal insects (purple arrows). The bat must be able to avoid interfering clutter echoes from the vegetation and potential jamming from the other bat's sound-echo pairs in order to detect, localize, and pursue the target insect it is hunting.

Although similar, the echolocation emissions of individual big brown bats appear to be distinct from one another (Masters and Jacobs, 1989; Masters et al., 1991). The characteristics of the echoes received by bats depend largely upon the characteristics of

the sonar signals they produce. A likely function of distinctive sonar signals is to reduce the probability that the sounds of other bats, which will vary slightly, will interfere with or jam the processing of echoes.

Big brown bats can successfully detect model echoes presented either forward or reversed in time, but their delay discrimination threshold worsens substantially for time-reversed echoes (Masters and Jacobs, 1989). Additional studies have reported decrements in delay acuity only when the time-frequency structure of model echoes (the organization of frequencies across the duration of the sound) had been altered (Surlykke, 1992; Masters and Raver, 2000).

Dramatic alterations in the time-frequency structure of echoes clearly affect perception of delay, but what about signals that are not as clearly unnatural as reversed echoes? How precisely must the received signal correspond to the hypothetical internal template? And how do slight differences in features of received echoes function to separate one bat's calls from another, or a target from the background clutter? The experiments in this dissertation explore the ability of big brown bats to reject clutter interference and avoid jamming and offer some possible explanations for how bats perform so well under acoustically challenging conditions.

**Clutter Rejection**

The presence of targets, such as insect prey, on substrates or in vegetation creates a complex acoustic scene for echolocating bats, with echoes from both the target and the clutter competing to be perceived (Moss and Surlykke, 2001). Clutter is a secondary target that returns additional echoes that can interfere with processing of the echoes of the

target of interest. For bats, filtering the target echo from the background clutter echoes takes place in several dimensions, such as direction (e.g., Sumer et al., 2009), frequency (e.g., Guillen-Servent and Ibanez, 2007), and time (e.g., Mohl and Surlykke 1989). Because both target echoes and clutter echoes are reflections of the emitted biosonar sound, they have a similar acoustic structure, differing mainly in strength and position (Simmons et al., 1988). Clutter echoes can impair the detection of target echoes due to masking when the echo of a target is concealed by stronger echoes from clutter targets, or when the target and the clutter are positioned in very close proximity so that their echoes arrive in the same time window.

Echoes of vegetation are among the most common echoes encountered by bats. Muller and Kuc (2000) and Yovel et al. (2008) demonstrated that different plant species could be classified based on both spectral and temporal features in their echoes. Yovel et al. (2009) extended this work by comparing the relation between the physical structure of different plants and the characteristics of their echoes. Acoustically, a plant can be approximated as a stochastic array of reflectors formed by its branches and leaves. The leaves reflect much of the energy of an emitted signal and the cross section of the leaves will strongly influence the spectra of the returning echoes. The spatial arrangement of the branches also is important because it defines the arrangement of the leaves. The authors completed a statistical analysis based on plant echoes acquired by a biomimetic sonar system. They determined that while echoes of all the plant species share a general temporal pattern, fine differences between echoes of difference plant species do exist and appeared to be due to the spatial characteristics of the plants (Yovel et al., 2009)

Several laboratory experiments have demonstrated that bats' ability to perceive targets is reduced by the presence of other echoes or noise pulses arriving within a time window around the arrival of the target echo. The critical interval of interference that results in decreased performance has been estimated to be less than 1 ms from the target (Joermann, 1984) to several ms from the target (McCarthy and Jen, 1983; Troest and Mohl, 1986). Troest and Mohl (1986) showed a significant masking effect resulted from increasing the speaker diameter in their phantom echo playback apparatus. Hartley (1992) also demonstrated the deleterious effect of clutter on target detection in a phantom echo experiment, and Simmons et al. (1988) demonstrated both backward masking and forward masking effects in experiments with big brown bats.

Studies like these give credence to the hypothesis that bats are not able to detect target echoes if they are masked by clutter echoes (Kalko and Schnitzler, 1993). But many recent studies show otherwise. For example, *Myotis nattereri*, a bat that uses short broadband FM signals, could detect prey in the clutter overlap zone while in flight (Siemers and Schnitzler, 2000). Successful target detection in clutter could depend on the presence of other acoustic cues. McCarthy and Jen (1983), for instance, found that bats detected a target in clutter more successfully when the target oscillated, which produced distinct spectral cues. Prey movement may also have served as a cue for the bats in Jensen et al.'s (2001) study, in which the bats were able to detect moths even when their echoes were overlapped with clutter echoes. However, even if bats use acoustic cues from their prey to help detect them in clutter, the bat's approach and capture must be guided at least in part by echolocation, if only to avoid collisions with the vegetation or

substrate itself. Indeed, under these conditions bats continue to emit echolocation sounds (Ratcliffe et al., 2005; Schmidt et al., 2000).

There are several behavioral strategies available to echolocating bats to reduce the detrimental effects of clutter. First, bats with flexible echolocation signals can select the signal type from their repertoire best suited for detection of a target in clutter. For example, big brown bats emit shorter and steeper FM sweeps with more bandwidth when foraging in clutter (Moss et al., 2006). Interspersing short interpulse intervals (IPIs) between longer interpulse intervals is another strategy used by bats in clutter, as it separates real targets from illusory phantom targets that are perceived due to ambiguity in assigning overlapping echoes to the correct broadcasts (Melcon et al., 2010). Variations in IPIs will allow bats to probe the environment far in front them while also avoiding nearby obstacles in their paths (Petrites et al., 2009). Second, the spatial separation of clutter and target is an important factor in reducing clutter interference. By increasing the amplitude of higher harmonics a bat can improve the spatial separation between target and clutter due to the frequency dependent increase in directionality of sound emission and echo reception (Sumer et al., 2009). Third, with increasing frequency, hearing becomes more directional and at higher frequencies the echolocation beam becomes more directional. Bats can use ear movements to position their ears into an optimal hearing position, and can move their emitted beam to create a favorable sound pressure level (SPL) ratio of target to clutter. Finally, bats may also simply fly at an optimal angle and distance in relation to target and clutter, choosing a position where spatial masking is least likely. Since the zone where clutter interference occurs increases with target

distance (Simmons et al., 1988), bats may maintain a shorter distance between themselves and their target during tracking.

Guillen-Servent and Ibanez (2007) report that the bat *Molossops temminckii*, which forages near background clutter, exhibits several unusual features in its echolocation calls. In the search phase, these bats used upwardly modulated FM calls. When approaching flying insect prey, the bats progressively increased the duration of their calls and interspersed downwardly modulated FM signals, which completely replaced the upwardly modulated calls at the end of the approach phase. The authors propose that upwardly modulated FM signals are an adaptation to foraging near clutter in a bat that is not particularly maneuverable. The broadband component of the signal might provide information for general spatial orientation and perception of the background, while the narrowband tail could serve for detection and possibly classification of prey (Guillen-Servent and Ibanez, 2007). Denzinger et al. (2001) came to a similar conclusion about the echolocation calls of another bat that forages near background clutter, *Barbastella barbastellus*. Their echolocation signals resemble those of *M. temminckii* in that the narrowband segment is at the higher frequency end of the FM sweep.

Other parameters of echolocation behavior are influenced by the presence or absence of nearby clutter. Moss et al. (2006) found that big brown bats altered the temporal emission pattern of their calls as they approached flying insects when the distance between prey and background was changed systematically. When the prey was close to a reflective background, the terminal buzz decreased in duration compared to the buzzes emitted when pursuing prey in an open room. In another experiment, big brown bats were flown in an open corridor between hanging plastic chains that created a dense,

distributed clutter (Petrites et al., 2009). The bats reacted to changes in the density of the clutter by emitting their echolocation calls in groups with more sounds and shorter intervals at higher clutter densities. The authors suggest that short interpulse intervals with groups of echolocation sounds serve to rapidly update perception of nearby objects, while longer intervals between groups of sounds may allow bats to probe farther into their surroundings to plan their future flight path. It is also possible that long intervals are used by the bat in an attempt to avoid pulse-echo ambiguity, a situation that occurs when echo streams from successive broadcasts overlap. The bats could be waiting for all the echoes from one sound to return before emitting the next sound to reduce confusion over which echoes correspond to which broadcasts. In situations such as this, it is essential that the bat be able to correctly assign the received echoes to the corresponding emitted calls because improper assignment of echoes would create spurious echo delay estimates and introduce "virtual objects" into the bat's delay images.

Melcon et al. (2010) present three possible methods to avoid ambiguity. One, bats can increase the pulse interval in the presence of clutter so that all echoes are returned before the subsequent call is emitted (Holderied and von Helverson, 2003). Two, bats can mark consecutive calls by alternating some parameter in a way that would contribute to echo assignment (Kingston et al., 2003). Three, alternation of long and short pulse intervals could prevent ambiguity by identifying virtual targets and real targets (Skolnik, 1970). A "virtual object" created by the incorrect assignment of an echo to a broadcast will stay at the same perceived position as long as the pulse interval is constant. Virtual echoes can be identified by alternating the pulse interval, which induces a range jitter of the virtual target but not of the real target (Skolnik, 1970). Melcon et al. (2010) tested

how *Myotis myotis*, an FM bat species, deal with ambiguous echoes when landing by positioning a background object that produced clutter echoes at 100 or 250 cm behind a landing grid. When the bats approached the grid to land, their broadcasts produced echo trains from the grid and the background object. The authors found no changes in signal structure, nor did they find any significant spectral cues in the echoes that the bats could have used to help disambiguate background clutter from landing grid. The bats did, however, alternate pulse intervals with clutter. In the presence of the background clutter behind the landing grid, bats decreased the number of pulses in the terminal buzz and changed the interval between pulses more often.

**Jamming Avoidance Responses**

The intensity and similarity of signals used by bats of the same species, as well as the social nature of most bats, opens up the possibility of detrimental interference. Animals that use active sensory systems, such as echolocating bats, face the potential problem of interference, or jamming, from the signals of conspecifics.

Echolocating bats possess many possible countermeasures to overcome jamming. First, the high directionality of the echolocation system (Schnitzler and Grinnell, 1977), and the directional sensitivity of the bat's ears (Grinnell and Schnitzler, 1977), enhances the bat's own echoes returning from straight ahead, and any sound coming from the side must be very intense to disturb echolocation (Suga et al., 1983). Second, a bat's biosonar creates an acoustical image of its surroundings that is derived from many successive echoes (Moss and Surlykke, 2001). The high redundancy of this system suggests that the loss of information by the masking of only a few of these echoes will have minimal

impact on performance. This might be especially true for bats that travel the same route from roost site to foraging grounds night after night (Rydell, 1989), because spatial memory may enable bats to orient with fewer echoes in familiar places (Griffin, 1958; Holler, 1995; Schmidt and Joermann, 1986). Third, time-sensitive neurons that respond to specific pulse-echo delays give many bats time windows of sensitivity to echoes. Finally, many echolocating bats appear to be versatile enough to alter parameters of their echolocation calls very rapidly in the presence of interference, demonstrating a behavioral jamming avoidance response (JAR). In many cases, individual bats demonstrate behavioral changes in their biosonar signals that result in the isolation of channels for active sensing and communicative behavior.

Some observational studies of multiple bats flying in the same air space have reported a change in timing or duration of biosonar emissions, presumably to avoid overlapping the sounds produced by nearby bats (Obrist, 1995; Surlykke and Moss, 2000). In field recordings of four species of vespertilionid bats (*E. fuscus*, *Euderma maculatum*, *Lasurisus borealis* and *L. cinereus*), all were observed to decrease their call duration and increase the interval between successive calls when conspecifics flew close by (Obrist, 1995). In another field recording of one of these species (*E. fuscus*), Surlykke and Moss (2000) observed that the interpulse intervals (IPIs) of bats flying in close proximity were less regular and both IPIs and call durations were shorter than those recorded from bats flying alone. These bats use short frequency-modulated sweeps, which might allow them to avoid temporal overlap by timing their vocalizations so that they fall in between those of other nearby bats. Temporally patterned calling, combined with a temporal analysis window, could be effective in ameliorating interference.

The more commonly observed behavioral change reported in bats flying near one another is a shift in call frequency. Big brown bats alter the timing of their calls and their frequency; both Obrist (1995) and Surlykke and Moss (2000) described how these bats lowered the lowest frequency end of their FM emissions. A strong correlation exists between signal duration and minimum frequency, but big brown bats appear to maintain control over their emitted frequency and can change the frequency of a vocalization without changing the duration (Bates et al., 2008; Surlykke and Moss, 2000). Sonograms of big brown bat calls indicate high variability in signal variables such as sweep rate and bandwidth, which can drastically influence the cross-correlation function between signal and echo. Different sonogram shapes will evoke different responses in a detector and therefore act as individual signal attributes (Obrist, 1995).

There are many more instances of frequency changes in multiple bat assemblages. When field recordings of pairs of *Tadarida brasiliensis* flying in close proximity are compared to virtual pairs (randomly matched individually flying bats), greater differences in peak frequency are found in the real pairs, with no significant differences in call duration (Ratcliffe et al., 2004). The recorded frequencies of *Tadarida teniotis* in an outdoor flight enclosure varied more when multiple bats flew together (Bayefsky-Anand et al., 2008). Differences were found in the peak frequencies of individuals of two species of Pipistrelle flying alone and the same individuals flying in a group of conspecifics; however, no differences were detected when an individual flew alone and in a mixed-species group (Bartonicka and Gaisler, 2007). Field recordings of *Balantiopteryx plicata* also revealed larger differences in peak frequency in bats flying in groups than in individuals flying alone (Ibanez et al., 2004). These analyses also showed a larger

variance for bats flying together, even though the sample size of recordings of bats flying alone was much larger than that of multiple bat recordings. Ibanez et al. (2004) observed a tendency toward an upward shift in the maximum frequency when bats were flying in groups, which could result from the bats treating the conspecifics as acoustic clutter, as some bats increase the frequency of their calls when approaching cluttered environments (Kalko and Schnitzler, 1993).

Ulanovsky et al. (2004) described two different types of JAR in groups of free-flying *Tadarida teniotis*. In symmetric JARs, a higher- and lower-frequency bat would each shift their frequencies upward and downward, respectively, maximizing the difference in frequency between the two individuals. In an asymmetric JAR, most often observed between closer conspecifics, only the upper-frequency bat altered its emissions, shifting its frequency upward. In contrast, the other species Ulanovsky et al. (2004) analyzed, *Taphozous perforatus*, showed no JAR at all. The authors hypothesize that the different spatial extent of the two species' sonar beams (the angle from which the bat picks up signals) could contribute to this interspecific difference. Sonar beam width is inversely proportional to the call frequency (Neuweiler, 2000). It is therefore possible that *T. perforatus*, which uses higher frequencies, has a narrower receiving beam and thus is less vulnerable to jamming than *T. teniotis*, which picks up jamming signals from a wider angle. The two bats may be using two different strategies, frequency shifts and spatial filtering, to achieve the same goal of jamming avoidance.

Some researchers have hypothesized that the calls of CF bats should be more susceptible to interference than the calls of FM bats (Haberstezer, 1981; Obrist, 1995; Schmidt and Joermann, 1986). CF calls do not provide the multiple acoustic

characteristics typical of FM sounds that may be used to distinguish them from another bat's calls. CF sounds also tend to be longer than FM sounds (10-100 ms), so overlaps between echoes and sounds are more likely. However, it has proven more difficult to jam the echolocation of CF bats than that of FM bats. Schmidt and Joermann (1986) reported that *Rhinopoma microphyllum* change the intensity of their emissions in the presence of noise, but do not shift their frequency. *Craseonycteris thonglongyai*, which produces short multiharmonic CF signals, does not alter its call frequency when flying among conspecifics (Surlykke et al., 1993). Jones et al. (1994) flew individual *Hipposideros speoris* and *H. fulvus* while playing back calls from other bats or calls from the test bat itself, and found no systematic change in emitted frequency. In another test of jamming in a Hipposiderid bat, Jones et al. (1993) flew three *Asella tridens* individually and then together to determine if the wide range of frequencies observed during group flight reflected attempts by the bats to alter their frequencies in order to reduce confusion from the echoes of other bats. The bats did not significantly alter their frequencies between individual and group flights, and the authors attributed the variation in CF frequency previously observed to variation associated with sex, age and size differences among bats (Jones et al., 1993). They proposed that each bat has a personal CF frequency, and because their hearing is most sensitive at that frequency, echoes returning to the bat at frequencies far outside this expected range could be ignored. It is not currently known how similar in frequency a sound would have to be to cause interference in a CF bat. However, Jones et al. (1994) reported a nonsignificant trend in which the highest collision scores occurred when a bat's own sounds were played back to them during obstacle avoidance. Although CF bats may appear to be more vulnerable to jamming at

first glance, the extremely narrowband tuning of the auditory neurons of CF bats to the bat's own frequency (Suga et al., 1987) make the possibility of spectral interference less likely. This is also a likely explanation for the absence of a JAR in bats such as *T. perforatus* (Ulanovsky et al., 2004), an FM bat that uses unusually narrowband calls. Although jamming a CF bat may be difficult, under certain circumstances sounds that are very similar to the bat's own emissions may have a deleterious effect. Demonstrating a JAR in CF bats may also be challenging because the narrow tuning of their acoustic foveae results in less versatility; changing the frequency of their emissions would result in echoes that do not possess the optimal frequency to which their auditory system is tuned.

If CF sounds are less amenable to frequency shifts, bats may instead choose to emit them only in certain contexts, as a study by Habersetzer (1981) suggests. In this experiment, *Rhinopoma hardwickei* was observed to employ FM emissions when leaving their roost in clusters, and CF emissions when they left singly. At the hunting grounds, bats flying in groups produced CF sounds separated into three distinct frequency bands, whereas bats flying individually used only the middle frequency band. These observations suggest that the CF echolocation calls are protected from interference by the bat, either by shifting its frequency in the presence of conspecifics or by switching to the use of broadband calls. FM sounds are typically shorter, which minimizes the overlap of the sound emission with the returning echo, enabling the bat to update more rapidly when the environment is changing quickly (i.e., when many other bats are flying nearby). Even in species that use primarily FM calls, narrowband, quasi-CF components may be treated specially to keep them free from interference. This portion of the call is relatively long in

duration, the lower frequencies it contains are less subject to atmospheric attenuation (Lawrence and Simmons, 1982), and playback of these lower frequencies produce the most effective jamming (Gillam et al., 2007). For example, *Pipistrellus pipistrellus* emit short FM sweeps ending with a CF portion, and they separate the CF portion of their calls by as much as 14 kHz when flying in groups (Miller and Degn, 1981). Bates et al. (2008) reported changes in the tail-end, nearly-CF component of the echolocation emissions of *E. fuscus*, another predominantly FM bat, during playback of CF tones. There appears to be conflicting evidence for a JAR in CF bats, but a more detailed survey of the circumstances under which they emit CF versus FM sounds may elucidate if and how these bats protect their calls from interference.

The majority of the studies discussed so far have been observational in nature, without active manipulation on the part of the researchers. Although observational studies provide important data on the natural behavior of animals, there are serious problems in trying to interpret them as either supporting or refuting the existence of a JAR in echolocating bats. First, in the field one cannot identify individual bats among several in a group flying together to determine how they might change their emissions under different conditions. Variations in frequency observed in the field may simply be due to individual differences in frequency among bats. Second, it is difficult to determine the extent to which changes in the emitted frequency might be due to Doppler shifts when recordings of flying bats are made from stationary microphones. Atmospheric attenuation and spherical spreading may also cause the higher frequencies of far bats to not be picked up by recording devices. Third, there are factors besides interference that cause bats to change their calls, such as foraging environment (Aldridge and Rautenbach, 1987;

Simmons et al., 1979; Simmons and Stein, 1980) and composition of the colony (Hiryu et al., 2006). Finally, outside of a controlled laboratory environment, it may be difficult to identify the task to which the bat is attending. They type of task in which it is engaged (e.g., searching for prey, social interaction) and at what point it is focusing its attention (and sonar beam) will affect the likelihood of its calls being susceptible to conspecific interference. Additionally, the spatial positions of the bats in field recordings are usually not known, so it is unclear if the directional biosonar beams of the bats are aimed towards each other (which may increase jamming) or away. Due to these factors, controlled experiments testing the JAR of bats are necessary to make conclusions about the role of interference in shaping biosonar emissions.

In a field playback experiment, Gillam et al. (2007) presented foraging *Tadarida brasiliensis*, which use short FM sweeps, with recorded echolocation calls at one of six frequencies. The bats exhibited higher frequencies in the presence of lower frequency playback, and most bats did not call at or near the playback frequency but tended to shift their frequencies higher. When Gillam et al. (2007) abruptly switched the stimulus as a bat approached the speaker, bats responded by shifting their frequencies upward, even when the playback was higher than their original frequency. This jumping above the playback frequency only occurred when the post-switch playback frequency was close to the bat's own emitted frequency. On average, bats shifted their call frequencies within less than 200 ms. Since the average IPI was 227 ms, this means that bats shifted their call frequency quite rapidly, in the first call emitted after the stimulus switch (Gillam et al., 2007). Experiments such as this one are valuable additions to the field observations of JARs in bats. The use of controlled stimuli certainly enhances our ability to draw

conclusions about what is actually occurring in the field; however, the most definitive results come from laboratory experiments.

Recently, Bates et al. (2008) performed a test of jamming in big brown bats in a laboratory setting. The bats were trained to walk down the arm of a Y-platform corresponding to the side on which they detected a target via echolocation, both in the absence and presence of a CF jamming tone which ranged from 18-32 kHz. All of the bats shifted the tail-end CF component of their emissions bidirectionally away from the jamming frequency only when it was within 3 kHz of their own preferred baseline frequency. There was no change in percentage of correct trials between jamming and non-jamming trials and the bats did not alter the durations of their sounds when shifting the frequency. Although all the bats tested exhibited the same response pattern, there was some individual variability in the point at which they shifted their frequencies and the degree to which they did so (Bates et al., 2008).

Two recent papers by Chiu et al. (2008, 2009) report potentially two different jamming avoidance responses made by pairs of big brown bats flying and competing for a tethered mealworm in a flight room. When two bats were flown together, differences in starting and ending frequency, duration, bandwidth and sweep rate significantly increased (Chiu et al., 2009). Pairs of bats with smaller pre-existing differences in call parameters showed more dramatic changes when flown together, as opposed to bats whose baseline echolocation calls were less similar, indicating an active change in call structure. Call design adjustments were also larger when the distance between the bats was smallest (Chiu et al., 2009). Using the same experimental setup and recording methods, Chiu et al. (2008) found another surprising strategy to avoid jamming in paired flights: one bat in the

pair would cease vocalizing for long periods of time (more than 200 ms). This silent behavior was related to the distance between the bats, the direction in which they were flying, and the similarity of their baseline echolocation calls. Since the silent bats showed no obvious decrement in flight performance (e.g., hitting obstacles), the authors propose that the bats may be using silence to avoid jamming while also listening to the sounds of their neighbors to orient.

**Plan of Dissertation**

In the first paper ("Jamming avoidance response of big brown bats in the field and the laboratory," unpublished), the echolocation calls of big brown bats were recorded in both the laboratory and the field. In the laboratory, pairs of bats were flown together in a flight room while each was wearing an onboard radio telemetry microphone to record its sounds. Parameters of the calls made by bats flying alone and bats flying with a conspecific were compared. In the field, a large array of ultrasonic microphones was used to record bats' vocalizations, which allowed triangulation of the specific position at which each call was generated. This method was contrasted with the use of a single microphone to record bats' sounds. Again, differences in signal structure were identified when bats were flying singly as compared to when they were near other bats. The results indicate that these bats actively change the frequencies of their calls in the presence of echolocating conspecifics, demonstrating a jamming avoidance response.

In the next experiment ("FM echolocating bats shift frequencies to avoid broadcast-echo ambiguity in clutter," Hiryu, Bates, Simmons and Riquimaroux, 2010 PNAS), big brown bats were again flown in a laboratory flight room while wearing radio

telemetry microphones, but this time individually, with rows of hanging plastic chains added to simulate dense vegetation, a source of acoustic clutter. Recordings of the bats' emissions show that they actively alter the frequency of their biosonar emissions at different points in the cluttered array, most likely to disambiguate long streams of echoes that resulted from each emission. Together, these two flight experiments demonstrate that big brown bats have similar behavioral adaptations for solving the problem of jamming from conspecifics and pulse-echo ambiguity from clutter. From here, I wanted to know the perceptual function of these small frequency shifts and how they allowed bats to segregate echoes into appropriate streams.

The final three experiments all used a psychophysical paradigm: a two-alternative forced-choice test using electronically generated echoes. Bats were trained on this set-up and asked to perform a simple ranging task as the electronically generated echoes that they received were manipulated. The third experiment ('Effects of filtering of harmonics from biosonar echoes on delay acuity by big brown bats (*Eptesicus fuscus*),' Bates and Simmons 2010 JASA) illustrates the effects of filtering out portions of returning echoes on delay acuity, and also demonstrates an effect of amplitude-latency trading. Bats required both FM1 and FM2 to be in temporal register for high delay acuity. Misalignment of the neuronal responses (caused by altering the amplitude of FM2) led to degraded acuity, but the removal of the entire FM2 caused little loss of acuity.

These results guided the next experiment, in which I used split-harmonic echoes to probe for the smallest temporal misalignment of harmonics perceived by bats ('Perception of echo delay is disrupted by small temporal misalignment of echo harmonics in bat sonar,' Bates and Simmons 2011 JEB). In this experiment, bats' delay

acuity for split-harmonic echoes was measured at various delays between the harmonics. When the relation between FM1 and FM2 was gradually disrupted through shifting the arrival time of the two harmonics, bats abruptly lost delay acuity through a process that amounts to defocusing of the image. Misalignments of FM2 with FM1 as small as 3 μs trigger the occurrence of full defocusing. This suggests that defocusing may be deliberately created in response to any deviation of echoes from the broadcast template.

After determining that misalignment of harmonics, or the neuronal responses to those harmonics, degraded delay acuity, and that a misalignment of as little as 3 μs was enough to disrupt delay acuity, I designed a final experiment to try to connect how this phenomenon served to help bats reject clutter ('Masking release by pulse-echo incongruities in bat biosonar,' Bates, Simmons and Zorikov, submitted to Science). These results, taken together with the previous papers, show how echolocating bats solve the difficult and important problem of eliminating clutter interference from background objects. The bats' solution is to blur or defocus biosonar images of clutter so that they no longer can mask perception of targets of interest located immediately to the front. Mechanisms at the highest levels of the auditory system segregate targets from clutter as an example of sharply defined categorical perception.

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CHAPTER 2

# Jamming avoidance response of big brown bats (*Eptesicus fuscus)* in the laboratory and the field

Bates, M. E., Knowles, J. M., Barchi, J. R., Simmons, J. A., Fujioka, E., Watanabe, Y., Furusawa, Y., Hiryu, S., and Riquimaroux, H. Jamming avoidance response of big brown bats (*Eptesicus fuscus*) in the laboratory and the field. In preparation.

## ABSTRACT

Echolocating bats face potential acoustical interference when flying and foraging near echolocating conspecifics. However, quantifying changes in the signal structure of echolocation emissions between bats flying alone and within a larger group has proven difficult. Here, we use two new methodologies, an onboard radio telemetry microphone and a multiple microphone array to record the sounds of big brown bats (*Eptesicus fuscus*) flying alone and in small groups both in the laboratory and in the field. In the lab, when two bats emitted sounds at a similar frequency, they bidirectionally shifted the frequencies of their emissions away from one another. If the pair started out with sounds of different frequencies (>5 kHz), they did not alter their sounds when flying together. Recordings from the microphone array in the field also show bats emitting sounds at many different frequencies, especially when flying in small groups. Both of these new methodologies provide support for a jamming avoidance response in big brown bats when flying with conspecifics under some conditions.

# INTRODUCTION

Echolocating bats depend upon information contained in the echoes of their ultrasonic emissions to identify, track and capture their prey, to avoid obstacles, and to perceive the arrangement of objects in the environment (Griffin, 1958). When flying or foraging in the presence of conspecifics emitting similar biosonar sounds, bats may experience interference from both the sounds and the echoes coming from other bats. Yet, videos of several bats hunting near one another, bats chasing each other, or even bats flying in large swarms show that bats seem to have no problem orienting and capturing prey with other bats in close proximity (Gillam et al., 2010; Simmons, 2005; Simmons et al., 2001).

It is widely expected that bats will alter some of the characteristics of their echolocation broadcasts to differentiate their own echoes from those of other, nearby bats. This type of jamming avoidance response (JAR) has been thoroughly studied in the electrolocation system of *gymnotiform* and *apteronotid* electric fish (Heiligenberg, 1991), and observational studies suggest that some bats may employ similar jamming avoidance strategies in the presence of conspecifics. There are reports of changes in timing or duration of biosonar emissions among multiple bats flying in close proximity, presumably to avoid overlapping of sounds (Obrist, 1995; Surlykke and Moss, 2000). There are many more reported instances of frequency changes in multiple bat assemblages (Bartonicka et al., 2007; Bayefsky-Anand et al., 2008; Gillam et al., 2007; Ibanez et al., 2004; Ratcliffe et al., 2004; Ulanovsky et al., 2004), which result in increasing the difference in emission frequency between two bats.

However, because much of these data supporting a JAR in flying bats has been observational, they are but rife with problems in interpretation. First, there are difficulties in identifying individual bats among several in a group flying together to determine how they might change their emissions under different conditions. Variations in frequency observed in the field may simply be due to individual differences in frequency among bats (REFERENCE). Second, it is difficult to determine the extent to which changes in the emitted frequency might be due to Doppler shifts when recordings of flying bats are made from stationary microphones. Atmospheric attenuation and spherical spreading may also cause the higher frequencies of far bats to be undetectable by recording devices. Additionally, the spatial positions of individual bats in field recordings are usually not known, so it is unclear if the directional biosonar beams of the bats are aimed towards each other (which may increase jamming) or away. Due to these factors, controlled experiments testing the JAR of bats are necessary to make conclusions about the role of interference in shaping biosonar emissions.

Two recent methodological developments have enabled researchers to record and interpret audio recordings of multiple bats with more certainty. The first of these is the Telemike, a radio telemetry microphone light enough to be worn by bats in flight (Hiryu et al., 2005; Hiryu et al., 2007; Hiryu et al., 2008; Hiryu et al., 2010). Telemikes have been used to record the sounds of bats performing a wide array of tasks in laboratory flight rooms. These devices are able to record bat sounds accurately with no Doppler shifts or loss of higher frequencies due to spherical spreading or attenuation. The second development is the use of multiple microphone arrays in both the field (Hiryu et al., 2008; Shiori et al., 2009) and the laboratory (Chiu et al., 2008, 2009). An array of

multiple ultrasonic microphones can be used to reconstruct the positions of bats at each sound that they emit, allowing one to track the paths of flying bats and correctly identify the number of bats in a recording and to which bat each emission belongs.

We used both of these approaches to examine the possibility of a JAR in big brown bats (*Eptesicus fuscus*) when flying with one or more conspecifics. In the first experiment, bats were flown singly and in pairs in a laboratory flight room while wearing telemikes and their sounds analyzed for any changes in spectrotemporal parameters such as frequency and duration. In the second experiment, bats in the field were recorded using a multiple microphone array and, for comparison, a single microphone. The data were reconstructed to identify instances of single bats and multiple bats foraging close to one another and were analyzed to look for changes in signal design between single bat flights and those involving multiple bats.

## EXPERIMENT 1: PAIRED AND SINGLE FLIGHTS IN THE LAB RECORDED WITH TELEMIKES

### MATERIALS AND METHODS

#### Subjects

Four adult female big brown bats (Nelly, Carmen, Edith and Olive) were used in this experiment. Bats were wild-caught in Rhode Island with a permit from the state's Department of Environmental Management. They were housed in individual cages at 22-23°C and 50% relative humidity with free access to food (mealworms; *Tenebrio* larvae) and vitamin-enriched water. The day-night cycle of the room was reversed at 12-h dark/12-h light so experiments could be conducted during the daytime. Body weight was

17-20 g. Experiments complied with Principles of Animal Care, publication #86-23 (1985) of the National Institutes of Health. Procedures were approved by the Brown University Animal Care and Use Committee.

**General Experimental Procedures**

The flight room (Fig. 1) measured 8.5 m (L) × 3.3 m (W) × 2.4 m (H). It was wrapped with copper mesh (40 threads/cm) to shield the room from external electromagnetic interference for the telemetry procedure. Walls and ceiling were covered with chemically-inert acoustically-absorbing panels (Sonex; Ilbrook Corp.) which damped background echoes. The sparse obstacle array was made of black plastic chains suspended vertically from the ceiling. Each chain was 2.4 m long, from ceiling to floor, with oval links 7 cm long and 4 cm wide, made from plastic rings 8 mm in diameter. All flights were done in complete darkness and were recorded by two synchronized thermal-imaging infrared video cameras (Indigo/"Merlin" midrange type with 25-mm lenses; FLIR Systems) that could "see" the bat from its own body heat as it flew along the room. The cameras were 1.4 m above the floor, separated by 2 m, and located outside the copper-mesh shielding so their internal electronics did not interfere electromagnetically with the telemetry recording. Video images were recorded at 30 frames/s on the video channel of a digital instrumentation data recorder (SIR-1000W; Sony Precision Instruments). Three-dimensional coordinates of the bats' flight paths were reconstructed from the stereo video recordings using commercial motion-analysis software ("Motus" v2.2; Peak Performance Technologies, Inc.).

Bats were released by an experimenter at the back of the room, between the two cameras, and allowed to fly without interruption until they chose to land. Between ten and twenty flights were completed each day, usually a combination of single flights and paired flights.

**Sound Recordings**

The bat's sonar pulses were recorded by a custom-made telemetry microphone (Telemike) mounted on the upper back and head. It registered the bat's FM broadcasts without Doppler shifts, amplitude changes, or spectral distortions using a small (3 mm diameter) omnidirectional Knowles FG-3329 electret microphone with response to above 100 kHz (i.e., beyond the upper limit of bat's hearing). After fur was removed from the bat's upper back and head by brief application of a depilatory cream ("Nair;" Church and Dwight Co.), the Telemike was attached with double-sided tape, with its microphone pointing forward, between the bat's ears and approximately 1 cm above the bat's mouth. The Telemike weighed less than 0.6 g, including the battery. The Telemike transmitted bat-sound-modulated radio signals with a carrier frequency between 90 and 105 MHz to an FM radio antenna (AM/FM amplified antenna; Radio Shack Corp.) attached to the ceiling of the flight chamber. The received signals were demodulated using a custom-made FM receiver, amplified and highpass-filtered at 10 kHz, then digitized at a sampling rate of 384 kHz (16-bit accuracy) and recorded on the Sony data recorder.

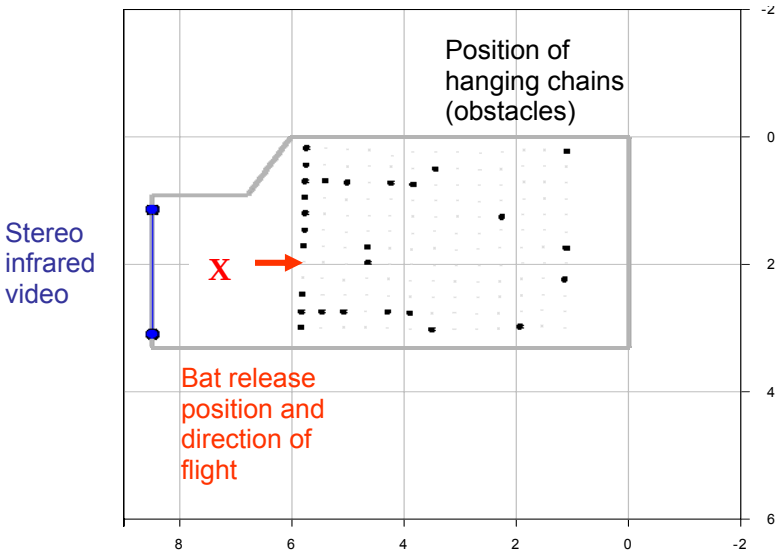


**Fig. 1.** Schematic of the laboratory flight room as viewed from above. The gray outline represents the border of the flight room, the two blue dots represent the stereo infrared video cameras, the red X marks the point where the bat or bats were released and the red arrow shows the direction they took into the array. The black dots represent the position of obstacles, hanging plastic chains that went from ceiling to floor. Bats typically entered the array and circled a few times before landing on a wall or on one of the chains.

**Data Analysis**

Each flight trial was digitized into a Pentium-3 computer using software supplied with the data recorder (Sony Real-Time and Streamer programs). The segment containing the flight, with the sounds from the Telemike(s), was clipped out and saved as a video file (.avi). Flight tracks of bats were reconstructed from the stereo video images using the video motion analysis software. Acoustic characteristics of the bat's sounds were analyzed by extracting the sound channels and displaying spectrograms using Adobe Audition v1.5 or spectrogram routines written in MatLab. Interpulse intervals (IPIs), durations, and beginning and ending frequencies of FM1 were determined from the spectrograms of individual Telemike sounds based on threshold at -25 dB relative to 0 dB peak amplitude.

Big brown bats emit low duty cycle frequency-modulated (FM) sweeps. The most prominent harmonic is the first (FM1) which typically sweeps downward in frequency from about 55 kHz to about 25 kHz. Although the FM sounds they emit cover a wide range of frequencies (from 20 to 100 kHz in three harmonics), there is a narrow range of frequencies at the tail-end of the FM sweeps that are emphasized for long-range target detection. These lower frequencies are less vulnerable to atmospheric attenuation in the air than higher frequencies, so they can penetrate through the air farther and still return as echoes audible to the bat. Several experiments have demonstrated that big brown bats alter the ending frequency of FM1 in the presence of clutter (Hiryu et al., 2010), interfering constant frequency tones (Bates et al., 2008) and conspecifics (Chiu et al., 2009; Surlykke and Moss, 2000). Therefore, ending FM1 frequency was one of the parameters examined for changes between single flights and multiple bat flights. We also measured beginning FM1 frequency, sound duration, and interpulse interval.

## RESULTS

### Pair 1: Nelly and Carmen

Fig. 2 shows the FM1 beginning frequencies used by Nelly and Carmen in both single and paired flights, while Fig. 3 shows the FM1 ending frequencies. In these box plots, the top and bottom of the box represent the 75$^{th}$ and 25$^{th}$ percentiles, the solid line in the middle represents the median, and the dotted line represents the mean. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles and solid circles are individual outliers.

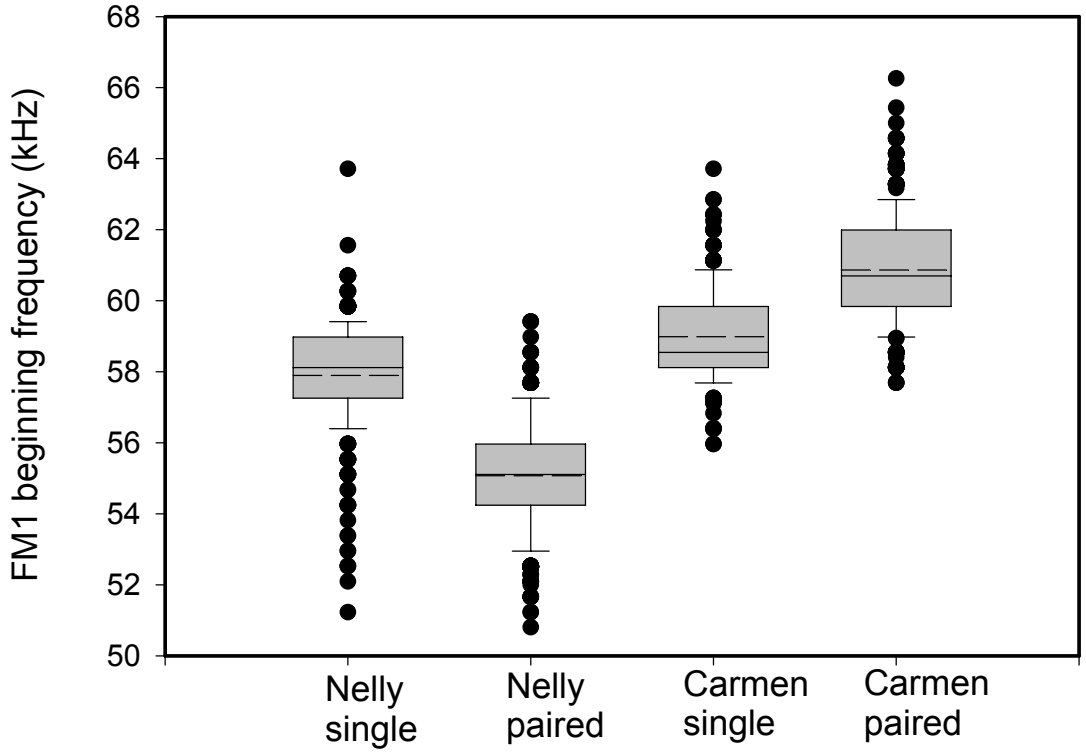


**Fig. 2** Box plots of the FM1 beginning frequencies used by Nelly and Carmen in single flights and in paired flights. Nelly lowered her mean FM1 beginning frequency by an average of 2.829 kHz when she flew with Carmen as compared to when she flew singly. Carmen increased her mean FM1 beginning frequency by an average of 2.656 kHz from single flights to paired flights with Nelly.

In single flights, both Nelly and Carmen emitted biosonar signals that were quite stable in beginning and ending FM1 frequency and in duration. The sounds of these bats were also very similar in frequency. For Nelly, a total of 440 emissions from 16 single flights were analyzed, while for Carmen, 261 emissions from 10 single flights were analyzed. In single flights, Nelly's mean FM1 beginning frequency was 57.8947 kHz (SD = 1.4958**)** and her mean FM1 ending frequency was 25.5046 kHz (SD = 1.0945) (Figs. 2, 3). In single flights, Carmen's mean FM1 beginning frequency was 58.2076 kHz (SD =

1.5730**)** and her mean FM1 ending frequency was 25.8969 kHz (SD = 0.8889) (Figs. 2, 3). Mean duration of Nelly's emissions in single flights was 2.08 ms (SD = 0.413), while Carmen's mean duration in single flights was 2.05 ms (SD = 0.426) (data not shown).

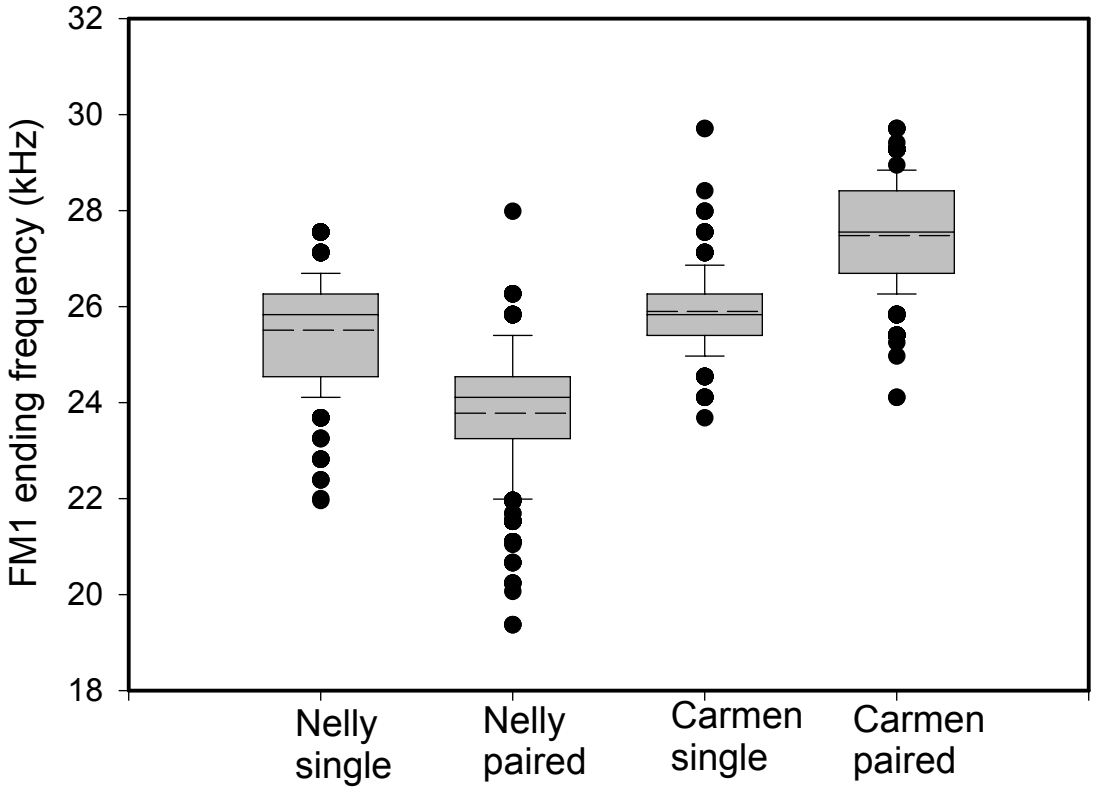


**Fig. 3** Box plots of the FM1 ending frequencies used by Nelly and Carmen in single flights and in paired flights. Nelly lowered her mean FM1 ending frequency by an average of 1.725 kHz when she flew with Carmen as compared to when she flew singly. Carmen increased her mean FM1 ending frequency by an average of 1.497 kHz from single flights to paired flights with Nelly.

From 27 paired flights, 1,372 emissions (823 from Nelly, 549 from Carmen) were analyzed. The FM1 beginning frequencies of both bats in paired flights are plotted in Fig. 2 and their FM1 ending frequencies in Fig. 3. Compared to her emissions when flying

singly, in paired flight Nelly shifted both her beginning and ending FM1 frequencies slightly lower, to a mean of 55.0658 kHz (SD = 1.4882) for FM1 beginning and a mean of 23.7799 kHz (SD = 1.5354) for FM1 ending. In paired flights, Carmen shifted her frequencies upward by a similar amount; her mean FM1 beginning frequency was 60.8640 kHz (SD = 1.4997) and her mean FM1 ending frequency was 27.3934 kHz (SD = 1.3993). The mean duration of sounds in paired flights was 2.14 ms (SD = 0.531) for Nelly and 2.08 ms (SD = 0.421) for Carmen (data not shown).

In their single flights, Nelly and Carmen emitted biosonar sounds with very similar beginning and ending frequencies. When flown together, Nelly tended to shift to slightly lower frequencies, while Carmen shifted her sounds upwards. The difference in frequency between a bat's emissions in single flight and paired flight were only 2-3 kHz, but when both bats shift it resulted in very little overlap in beginning and ending frequencies. The mean difference between Nelly and Carmen's FM1 frequencies (both beginning and ending) was approximately 0.3 kHz in single flights; in paired flights the difference increased to 3.6-5.8 kHz.

**Pair 2: Edith and Olive**

Again, the beginning and ending frequencies and durations used by both bats when flying singly remained stable. Edith and Olive, however, demonstrated pre-existing differences in biosonar emission frequency during their single flights. For Edith, a total of 171 emissions from 6 single flights were analyzed, while for Olive, 111 emissions from 5 single flights were analyzed. Edith's mean FM1 beginning frequency in single flights was 56.9936 kHz (SD = 1.8318**)** and her mean FM1 ending frequency was 23.9634 kHz (SD

= 0.9777) (Figs. 4, 5). In single flights, Olive's mean FM1 beginning frequency was 61.1216 kHz (SD = 1.1707**)** and her mean FM1 ending frequency was 27.7296 kHz (SD = 0.6656) (Figs. 4, 5). Mean duration of Edith's emissions in single flights was 2.12 ms (SD = 0.373), while Olive's mean duration in single flights was 2.11 ms (SD = 0.366) (data not shown).

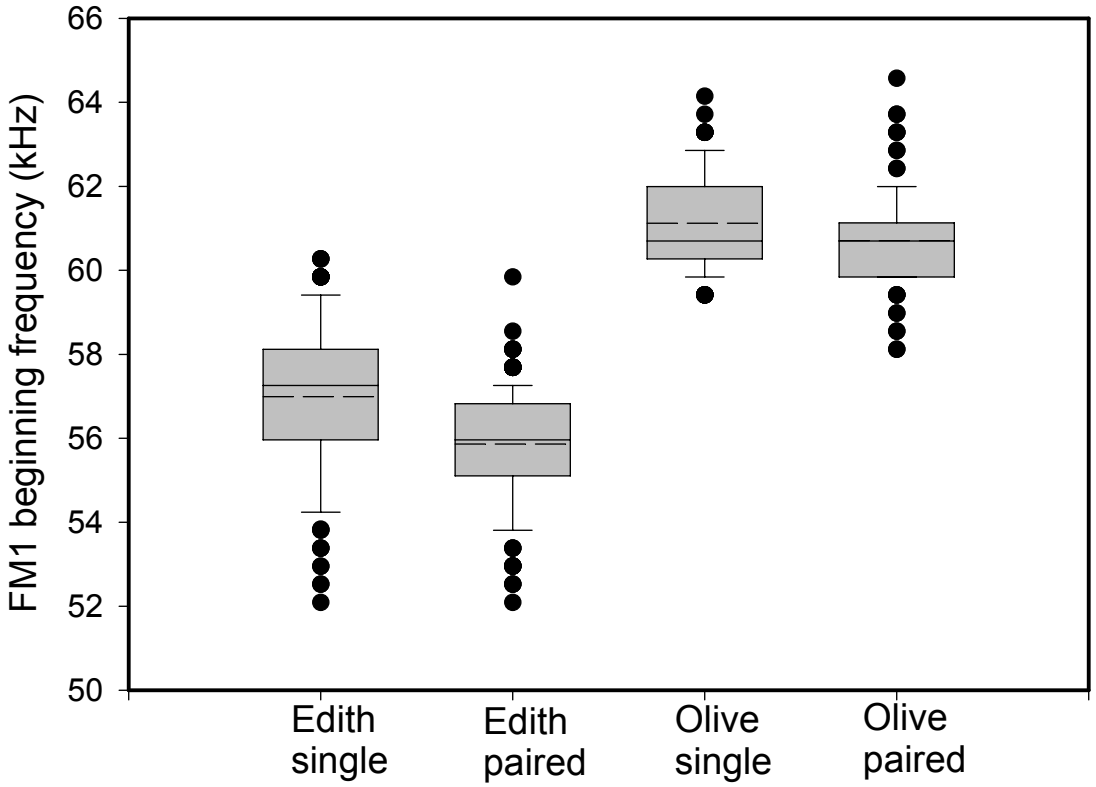


**Fig. 4** Box plots of FM1 beginning frequencies used by Edith and Olive in single and paired flights. These two bats did not alter their beginning frequencies very much when they flew together, as compared to when they flew singly. The mean difference in Edith's FM1 beginning frequency between single and paired flights was 1.132 kHz, while for Olive it was 0.419 kHz.

From 10 paired flights, 370 emissions (248 from Edith, 122 from Olive) were analyzed. Unlike the previous pair of bats, Edith and Olive did not alter the frequency of their emissions significantly when they flew as a pair as compared to when they flew singly. Edith's mean FM1 beginning frequency in paired flights was 55.8613 kHz (SD = 1.3348) and her mean FM1 ending frequency was 23.6856 kHz (SD = 1.0609) (Figs. 4, 5). Olive's mean FM1 beginning frequency in paired flights was 60.7027 kHz (SD = 1.0965) and her mean FM1 ending frequency was 27.3466 kHz (SD = 0.6436) (Figs. 7, 8). The mean duration of sounds in paired flights was 2.09 ms (SD = 0.347) for Edith and 2.15 ms (SD = 0.386) for Olive (data not shown).

In their single flights, Edith and Olive demonstrated pre-existing individual differences in beginning and ending FM1 frequencies. When flown together, these differences remained largely the same; on average, neither bat shifted its frequencies more than about 1 kHz between single and paired flights. The mean difference between Edith and Olive's FM1 frequencies (both beginning and ending) was 3.7-4.1 kHz in single flights; in paired flights the difference was 3.8-4.8 kHz.

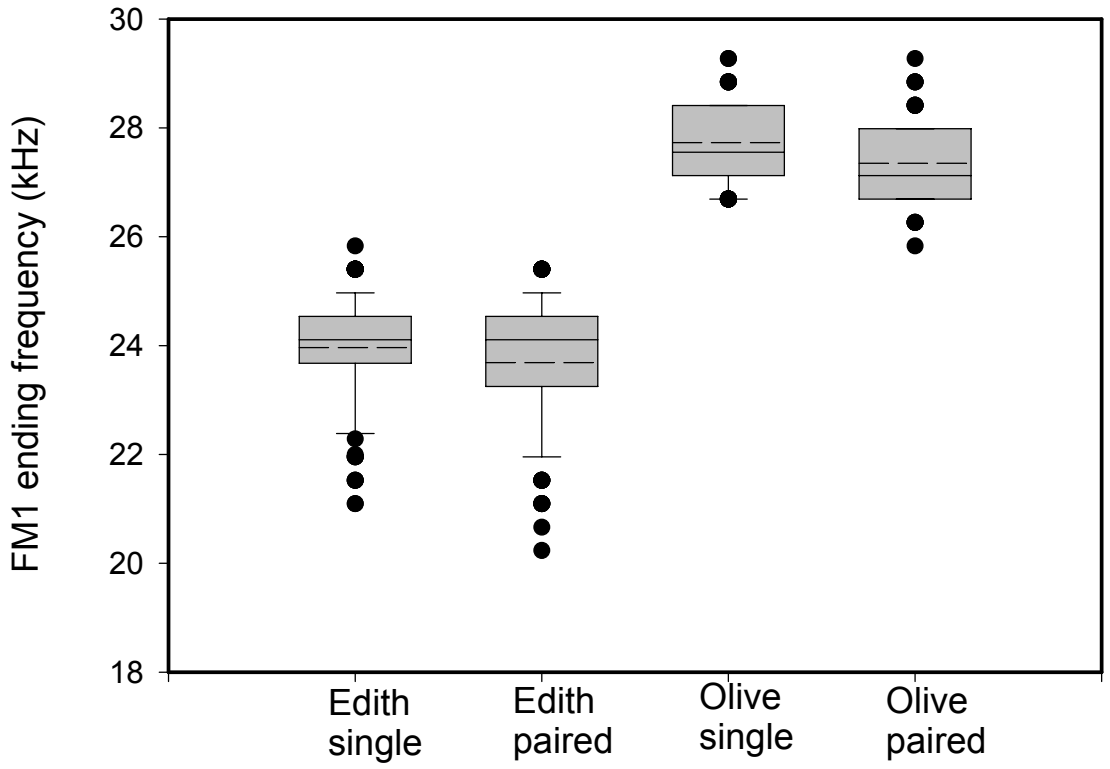


**Fig. 5** Box plots of FM1 ending frequencies used by Edith and Olive in single and paired flights. These two bats did not alter their beginning frequencies very much when they flew together, as compared to when they flew singly. The mean difference in Edith's FM1 ending frequency between single and paired flights was 0.279 kHz, while for Olive it was 0.383 kHz.

## EXPERIMENT 2A: SINGLE AND MULTIPLE BATS IN THE FIELD RECORDED WITH A SINGLE MICROPHONE

### MATERIALS AND METHODS

**Recording Site**

The field site was an open field surrounded by vegetation, including dense, tall trees, in a rural area of Rhode Island. Recordings were begun shortly after sunset after

bats had emerged from their roost in a nearby attic and were foraging over the open field. This recording session, from August 2008, lasted approximately two hours.

**Sound Recordings**

A hand-held ‘‘Merlin’’ midrange infrared thermal imaging camera (Indigo Systems, Inc.; spectral sensitivity X–Ymm) was used to watch and make video recordings of bats in flight. The bats’ sonar sounds were picked up by ultrasonic condenser microphones (Titley Electronics, Ltd.) operating with an Aco-Pacific microphone power supply, amplified with a Reson model VP-2000 amplifier/filter (3-dB points set at 10–100 kHz), and recorded along with the infrared video on a Sony wideband SIR-1000W digital instrumentation recorder with a SVB-10 video board. This system could record video and audio continuously for up to 60–90 min. Most of the present observations were made of bats at distances of 5 to 25 m using a 25-mm lens (13 magnification) or a 13-mm lens (0.53, or slightly wide angle). Time registration was achieved by writing SMPTE time/frame counts onto the video frames of both video recordings using battery-powered Hirata VG-50 and TRG-50 coding units.

**Data Analysis**

Segments of interest (those that contained good quality calls of at least one bat) were downloaded as binary files and MPEG video clips into a Pentium-3 PC using software supplied with the digital instrumentation recorder. Audio files were broken into segments and categorized by two raters as either containing sounds from a single bat or from multiple bats (Fig. 6). The raters used the accompanying video, when available, to

confirm the number of bats in a given segment of recording. If video was unavailable or unclear, the presence of multiple bats in an audio segment was identified by differences in sweep rate, sweep shape, and changes in interpulse interval (IPI; remains highly consistent within bats during search phase). Only segments that both raters categorized the same were analyzed.

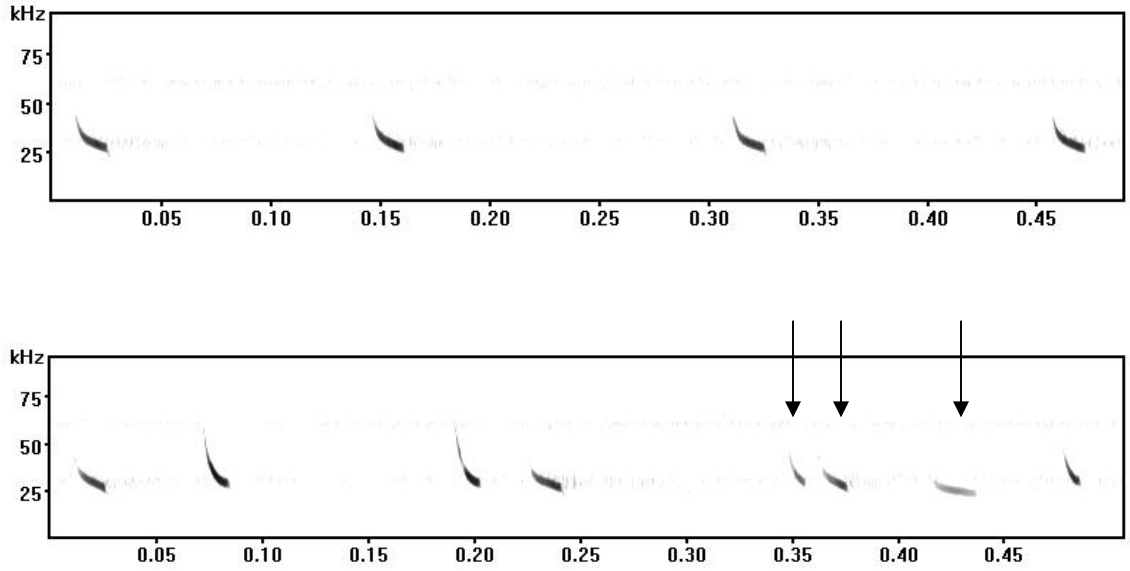


**Fig. 6** Examples of biosonar sounds recorded in the field with a single ultrasonic microphone. (*A*) A section of recording from a single *E. fuscus*. (*B*) A section of recording in which three *E. fuscus* were flying in close proximity. Black arrows indicate different bats.

A custom-written MATLAB routine was used to measure the deviation of each sound from the mean of the segment to which it belonged. This was performed for two parameters, duration and ending FM1 frequency. Due to lowpass environmental filtering, beginning FM1 frequency could not always be reliably obtained from all the emissions and so was not analyzed in these data.

RESULTS

All of the segments analyzed contained the sounds of one to four bats. The broadcasts of bats in groups of only a few conspecifics did not often overlap, most likely due to the timing and wide-spacing of vocalizations. The duration of sounds was very similar between flights with one bat and flights with multiple bats (single bats: N=858, mean duration = 0.01022 s, SD=.004088; multiple bats: N=939, mean duration = .01045 s, SD=.004509; data not shown).

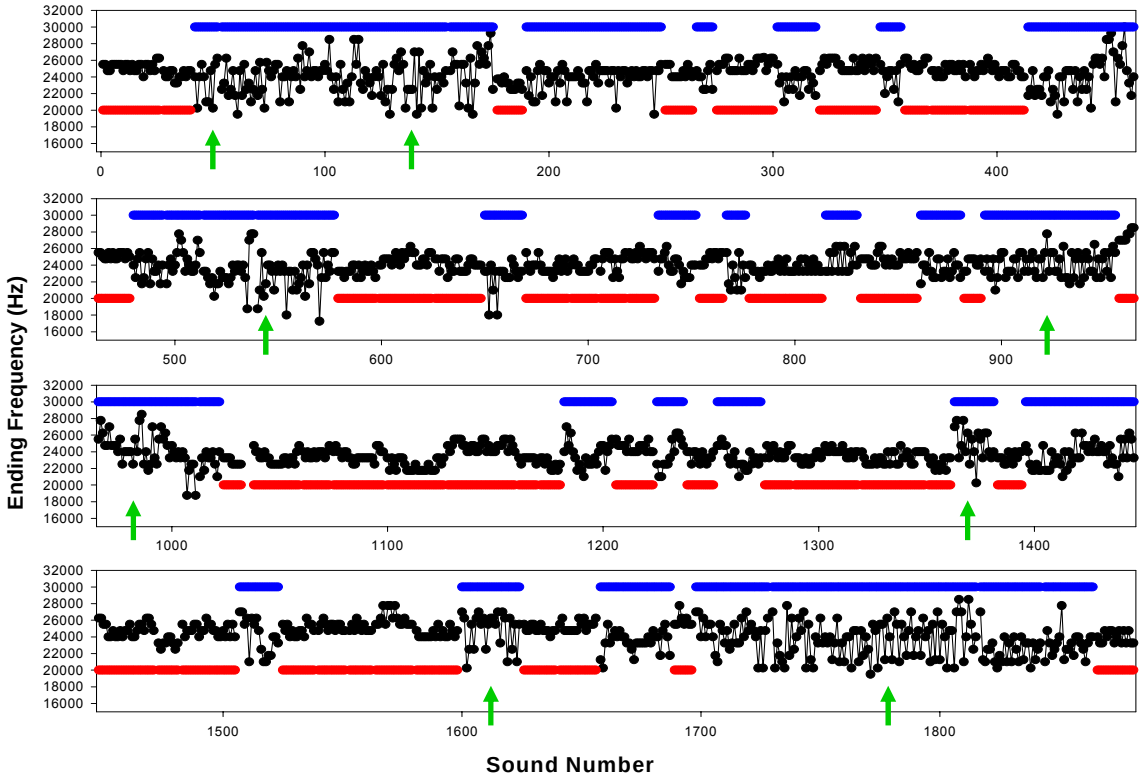


**Fig. 7** Ending frequencies of free-flying *E. fuscus*. Segments containing one bat are indicated by a red line below the data; segments containing multiple bats are indicated by a blue line above the data. Take note of the jumps in frequencies between successive sounds that occur often in multiple bat segments (green arrows).

When small numbers of bats flew close together, the variability in ending frequency was greater than expected. Fig. 7 plots the ending frequency of every bat sound analyzed from the recording. Segments containing the sounds of a single bat are indicated by a red line below the data, while segments containing the sounds of two or more bats are indicated by a blue line above the data. Qualitatively, there is more variability in ending frequency in the multiple bat segments (some of the areas with especially conspicuous jumps in frequencies between successive sounds are labeled with green arrows).

A specially-written MATLAB routine was used to calculate the deviation of each sound from the mean of the segment from which it came. Fig. 8 shows the ending frequency of the sounds plotted in Fig. 7 as deviations from the means of their segments. The top plot shows the deviation from the mean for sounds made in single bat flight situations, while the bottom plot shows the much greater deviations present in ending frequency when multiple bats are flying close together. Together, both of these figures offer qualitative evidence that there is more variability in ending frequency when multiple bats are flying together than when bats are flying singly.

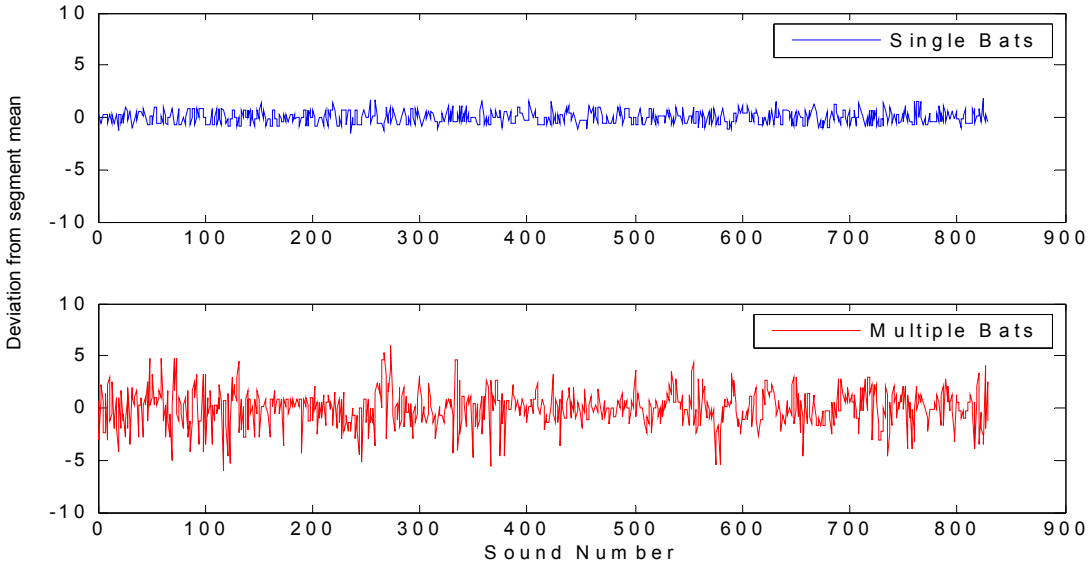


**Fig. 8** The ending frequency of sounds plotted as the deviation of each sound from the mean of the segment from which it came. The top plot shows the deviation from the mean for sounds made in single bat flight situations; the bottom plot shows the much greater deviations present in ending frequency when multiple bats are flying close together.

## EXPERIMENT 2B: SINGLE AND MULTIPLE BATS IN THE FIELD RECORDED WITH A MULTIPLE MICROPHONE ARRAY

## MATERIALS AND METHODS

### Recording Site

Recordings were conducted at the corner of a large sod field in Kingston, RI. The site was surrounded on two sides by large trees and dense vegetation. Recordings were conducted on three nights in July and August 2010. On each session, recordings began shortly after sunset and continued for approximately two hours.

### Sound Recordings

On each recording day, two array towers were erected approximately 8 m apart, each supporting four microphones arranged in a star shape separated by 0.9 m. Echolocation signals were recorded using eight ultrasonic microphones (423-1064-ND Miniature Electret Microphone; Knowles Electronics, Itasca Illinois) mounted on custom-built amplifier and power supply boards. The three-dimensional positions of the eight microphones were measured with millimeter precision using a surveying station equipped with a laser range finder (Series30R Total Station; Sokkia, Kanagawa Japan). The eight channels of audio were digitized at 192 kHz and recorded to tapes using the Sony SIR-1000W digital recorder. During the recording session, operators listened to a separate bat detector (D230; Pettersson Elektronik, Sweden) and triggered the recorder whenever bats were audible on the detector. After the onset of recording, recording lasted for 2 minutes or until the bat activity diminished.

**Data Analysis**

Data were downloaded from the recording tapes as binary files and transferred to an Apple computer. Data were analyzed using a custom written MATLAB routine. Bat calls were located on each of the eight channels using an energy detector. Each bat call was localized in three dimensions by calculating the time difference of arrival between each channel and a reference channel on each of the array towers. Time differences of arrival were passed to a passive localization algorithm based on a linear solution of time difference of arrival (Gillette and Silverman, 2008). After localizing the sounds, two analysis methods were performed. First, videos were produced which simultaneously displayed a three dimensional animation of the localized coordinates and spectrograms of

the sound recordings from one of the channels. A manual rater used these animations to separate calls from the two bats, then recorded parameters of the calls by displaying the same data in Adobe Audition. The rater was instructed to break the data into segments short enough to be confident that the identity of multiple bats could be maintained.

## RESULTS

Fig. 9 shows the results from one 14 s segment of recording. First, time-of-arrival differences of calls at the multiple microphones were used to determine the position of the calling bat at each call. This revealed two distinct flight paths (black and red paths in Fig. 9A) corresponding to the two vocalizing bats present in the recorded segment. A hand analysis of all the bat emissions resulted in assigning each call to either bat 1 or bat 2, and a listing of the recorded acoustic parameters for each bat's sounds. In Fig. 9B, histograms plot these parameters (maximum frequency, minimum frequency, centroid frequency and duration) for each bat (again, in red or black). The two bats overlapped quite a bit on sound duration, and slightly on maximum frequency. They were most easily separated with the two parameters minimum frequency and centroid frequency. Fig. 9C shows the assignation of calls to either bat 1 or bat 2 made by the hand analysis and by the automated computer program. In this segment, only one call was not assigned to the same bat by both human and program. In the lower portion of Fig. 9C is the computer program's assigned calls for the two bats (black and red) plotted in terms of the three frequency parameters. The two bats are separated into two clearly visible clusters with very little overlap. In this case, hand analysis and automated analysis yielded the same results.

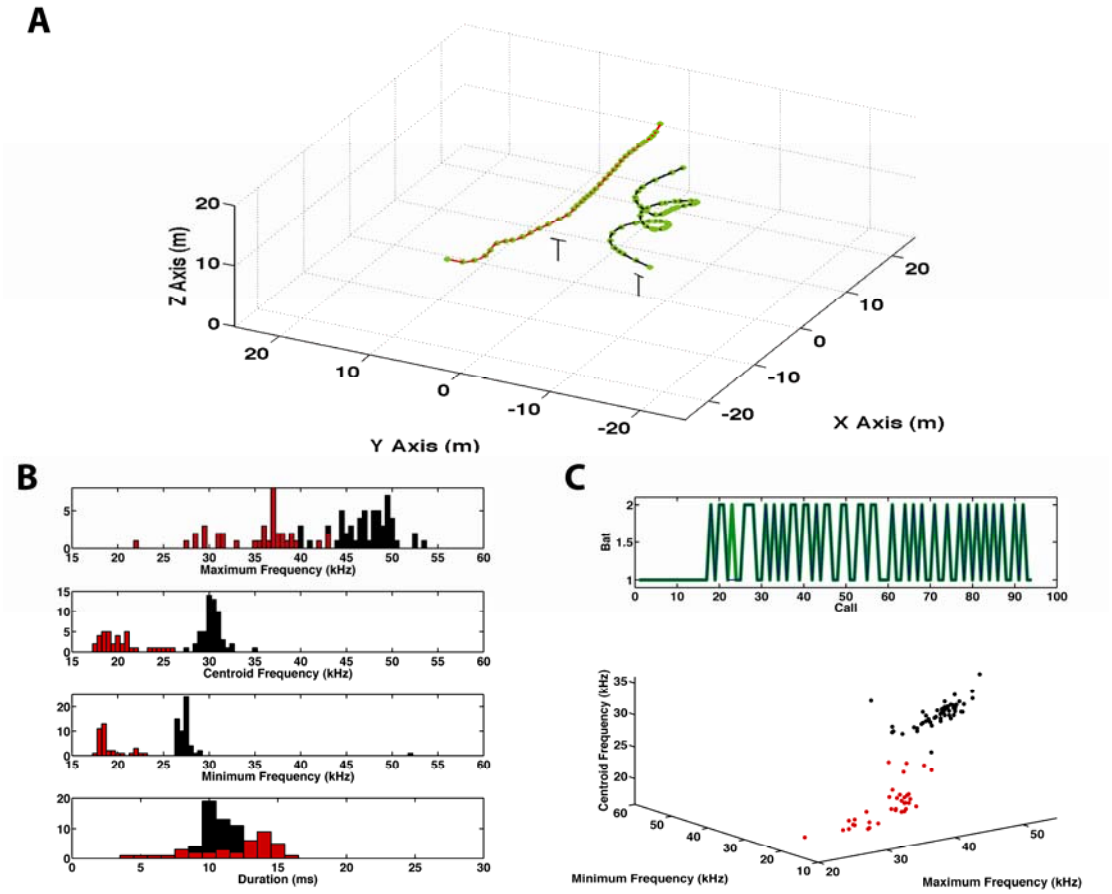


**Fig. 9** Reconstructed flight paths of two bats and comparison of hand analysis with automated analysis. (*A*) The flight paths of two bats, labeled in red and black, during a 14 s segment of recording. The black Ts represent the two microphone arrays. These paths were reconstructed based on the time-of-arrival differences at the microphones. (*B*) Results of the hand analysis for four acoustic parameters: maximum frequency, centroid frequency, minimum frequency, and duration. Red bars correspond to the bat with the red flight path in (*A*); black bars to the bat with the black flight path in (*A*). The bats were most easily separated using centroid and minimum frequency. (*C*) Top: Comparison of call assignations made by hand analysis (green) to those made by an automated MatLab program (blue). There was only one call which was not assigned to the same bat by both methods. Bottom: Three-dimensional plot of calls emitted by the two bats (red and black) grouped into clusters based on three acoustic parameters: centroid, minimum, and maximum frequency.

# DISCUSSION

The data collected above using three different experimental paradigms all show the same pattern: under circumstances in which jamming is likely, big brown bats alter the frequency of their emitted biosonar sounds in a jamming avoidance response. In the past, evidence for a JAR in echolocating bats has been elusive, due in part to the limitations of recording devices. Here, we show that data obtained with an on-board radio telemetry microphone in a laboratory flight room and with a multiple-microphone array in the field yield the same results as single microphone recordings in the field. These new techniques are valuable, however, in that they allow more certainty of the number and positions of the bats involved and the acoustic parameters of their emitted sounds.

## Jamming Avoidance in the Laboratory

Two pairs of big brown bats were flown in a laboratory flight room while each was wearing a Telemike to record its sounds. In the pair of bats that emitted sounds of similar frequency when flying alone, both bats exhibited a JAR, one shifting its first harmonic slightly upward in frequency and the other shifting slightly downward. However, in the pair of bats with a larger separation between their emitted frequencies in single flights, neither bat altered its sounds when flown together. This suggests that certain, similar sounds are more likely to cause interference for echolocating bats and that an active change in their signal structure may not be necessary in the presence of all conspecifics, but only ones with the most similar sounds and hence the greatest potential for jamming.

The Telemike allows observation of emitted biosonar sounds and precise measurement of acoustic parameters without problems such as the directional properties of the recording microphone and the emitted sound, or traveling loss of the sound in air (e.g., Boonman and Jones, 2002; Hartley et al., 1989; Lawrence and Simmons, 1982). Hiryu et al. (2010) used the Telemike to record small changes in the frequency of big brown bat broadcasts when the bats were flown in a densely cluttered test room. The clutter consisted of rows of hanging plastic chains, which presented the bats with many objects in depth. Hiryu et al. (2010) reported that when the bats encountered pulse-echo ambiguity, due to the returning echoes from one emission overlapping the returning echoes from the subsequent emission, they shifted the frequencies of proximal sounds to segregate the two echo streams. This behavioral response (shifting broadcast frequency by a small amount) is very similar to what is observed when multiple bats are flown together (e.g., Bartonicka et al., 2007; Bayefsky-Anand et al., 2008; Gillam et al., 2007; Ibanez et al., 2004; Ratcliffe et al., 2004; Surlykke and Moss, 2000; Ulanovsky et al., 2004). In essence, the bats in Hiryu et al.'s (2010) experiment are avoiding jamming, but from their own reverberant echoes and not from the sounds of conspecifics.

One alternative to an on-board microphone is the use of a multiple microphone array in a laboratory test room. Ghose and Moss (2003) successfully estimated the direction of big brown bats' "sonar gaze" by comparing signal intensity across an array of microphones to deduce the direction in which the bat aims its sounds. Bats were tracked as they avoided obstacles and searched for tethered insect prey in a flight room fitted with an array of 16 microphones positioned in a U-shape along three of the room's walls. This

microphone array made it possible to reconstruct the sonar beam pattern of flying bats as they performed realistic tasks in the laboratory.

A similar set-up (flight room with a 16-microphone array) was used to directly investigate the ways bats change their sounds when flying in close proximity to a conspecific (Chiu et al., 2008; Chiu et al., 2009). These two papers report potentially two different jamming avoidance responses made by pairs of big brown bats flying and competing for a tethered mealworm in a flight room. When two bats were flown together, differences in starting and ending frequency, duration, bandwidth and sweep rate significantly increased (Chiu et al., 2009). Pairs of bats with smaller pre-existing differences in call parameters showed more dramatic changes when flown together, as opposed to bats whose baseline echolocation calls were less similar, indicating an active change in call structure. Call design adjustments were also larger when the distance between the bats was smallest (Chiu et al., 2009). Using the same experimental setup and recording methods, Chiu et al. (2008) found another surprising strategy to avoid jamming in paired flights: one bat in the pair would cease vocalizing for long periods of time (more than 200 ms). This silent behavior was related to the distance between the bats, the direction in which they were flying, and the similarity of their baseline echolocation calls. Since the silent bats showed no obvious decrement in flight performance (e.g., hitting obstacles), the authors propose that the bats may be using silence to avoid jamming while also listening to the sounds of their neighbors to orient.

We found a similar response as Chiu et al. (2009): in pairs of bats with similar echolocation emissions, bats appear to actively change signal parameters to decrease the similarities of their calls. This doesn't appear to be necessary in bats whose calls start off

dissimilarly. In our experiment, we found no evidence of prolonged periods of silent flying. This could be due to differences in experimental set-up. The bats in our study, unlike those in Chiu et al.'s (2008), were not performing any particular task. They were circling the flight room, which was arranged with a sparse array of hanging chains as obstacles, but they were not tracking a reward or competing with the other bat for a reward. Perhaps the presence of slightly more obstacles, or the absence of competition for a food reward, created a situation that did not lend itself to this type of jamming avoidance. Similarly, if jamming avoidance by shifting frequency is only seen in pairs of bats emitting similar sounds, one would also expect jamming avoidance by ceasing vocalizations to be exhibited by bats of similar frequency. We only analyzed one pair of bats that exhibited a JAR. Perhaps a study of more pairs, in a broader range of flight situations, would turn up more jamming avoidance strategies.

**Jamming Avoidance in the Field**

In this paper, we present examples of single bat and multiple bat flights recorded in the field with a single microphone for comparison to the results obtained when recording with multiple-microphone arrays. There are two main concerns when recording bats in the field with a single microphone. One, due to the high directionality of bat calls and the effects of spherical spreading, atmospheric absorption, and Doppler shifts, many of the acoustic parameters of interest become attenuated or otherwise warped by the time they reach the microphone. Single microphones also provide no information on the position of the bat and its distance and flight direction, which can all affect the quality and content of the recording. Microphone arrays do not solve the problem of natural

acoustic filtering effects, but the presence of multiple microphones increases the likelihood that the bat will be close to one of them so that the parameters can be obtained cleanly on at least one recording channel. Also, by measuring differences in time-of-arrival of a sound at all the microphones, the bats' position and distance can be estimated. Two, when the calls of multiple bats are present in a single recording segment, a single microphone offers no way to separate them beyond using acoustic parameters such as interpulse interval and differences in sweep shape and rate. If the bats are emitting sounds of similar frequency and bandwidth, this can be very difficult. Reconstructing the flight paths of multiple bats using a microphone array makes assigning calls to one bat or another much easier.

Ulanovsky et al.'s (2004) study illustrates the difficulties of separating the calls of multiple bats from a single microphone recording. Ulanovsky et al. (2004) recorded with a single bat detector the echolocation calls of two bat species during foraging. To separate the calls of conspecific bats in the same recording period, they first looked at two acoustic parameters, the lowest frequency and the interpulse interval. The researchers report that the emissions of individual bats were clearly distinguishable by the consistency of these two parameters. They chose recorded segments which contained only two bats, each with prominent echolocation vocalizations, for analysis, and used a combination of lowest frequency versus duration plots and histograms of interpulse intervals to separate the calls of pairs of bats in the same airspace.

Small multiple-microphone arrays have been used in the field to calculate the source level (Surlykke and Kalko, 2008) and beamwidth and directionality (Surlykke et al., 2009) of bat echolocation emissions. In both experiments, the researchers used a

linear microphone array with three microphones to record the bats' sounds. From the array recordings, the flight paths were reconstructed, making use of time-of-arrival differences between the microphones. From the time-of-arrival differences, the researchers estimated the bat's position for each call. To determine the source levels of echolocation calls, Surlykke and Kalko (2008) needed to know more than just the position of the bats. They employed stereo-photography along with the three-microphone array to determine the distance, direction and flight path of the bats in their study. Due to the high directionality of bat calls, they only analyzed recordings of bats that were directly approaching one of the microphones.

In the present study, we obtained similar results from the single microphone recordings and the multiple microphone recordings; i.e., bats flying in the presence of conspecifics had more variable ending frequencies than bats flying alone. However, the multiple microphone array is superior in that it allows more confidence in the assignments of calls to certain bats. The array also provides us with much more information on the bats' flight paths and their specific position in relation to the microphones. This information is helpful in determining which bats are making which sounds, but also for observing how multiple bats forage and fly near one another. We are able to see if bats interact with one another on the wing while foraging as well as how such closeness may affect their emitted sounds. Multiple microphone arrays lend themselves to automated analysis which, as we show here, yields data similar to that of a single microphone recording, but augmented by additional information about the bats' positions and paths.

Another difficulty in quantifying a JAR in echolocating bats is the dynamic nature of their vocalizations. Many bats are highly flexible and able to adapt their signals to suit different tasks and environmental conditions. For instances, bats in flying in enclosed spaces like laboratory flight rooms have been shown to emit calls of shorter duration, larger bandwidth and lower intensity than in open spaces (e.g., Surlykke and Moss, 2000). Though some studies show that bats actively avoid areas high in ambient ultrasonic noise, like running water or vegetation rustling in the wind, (Schaub et al., 2008; von Frenckell and Barclay), others suggest that bats change acoustic parameters of their emissions in the presence of such noise (e.g., chorusing insects; Gillam and McCracken, 2007). Different flight situations also call for different echolocation signals. In more cluttered habitats, many bats increase the bandwidth of their emissions to obtain more precise information concerning target location (Simmons and Stein, 1980). The bat *Balantiopteryx plicata* emits search phase calls of greater bandwidth when foraging among trees than when in open spaces (Ibanez et al., 2004). When the sounds of pipistrelles in different flight conditions (emerging from the roost, commuting to and from the foraging site, foraging, and returning to the roost) were compared, researchers found that duration, pulse interval, highest frequency and bandwidth all differed significantly (Berger-Tal et al., 2007). Brazilian free-tailed bats emerge from their caves in a tight, densely-packed column, which the bats seem to treat as a highly cluttered habitat (Gillam et al., 2010). Their calls when emerging are shorter and of higher frequency and broader bandwidth than the calls they emit foraging in open areas. Again, these types of signals provide more accurate estimates of target position and a shorter maximum detection distance and so are suited for cluttered habitats.

Separating and analyzing the calls of multiple bats in the same airspace has been a challenge for researchers interested in the possibility of a JAR in bats. In this study, we discuss the results obtained with two technologies, an on-board radio telemetry microphone and the use of multiple microphone arrays, for recording the calls of bats in the laboratory and the field. Both of these techniques provide support for a JAR in bats; specifically, an active alteration of frequency in the presence of conspecifics emitting similar sounds. Further studies will add to our knowledge of when such jamming avoidance strategies are implemented.

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CHAPTER 3

## FM echolocating bats shift frequencies to avoid broadcast-echo ambiguity in clutter

Hiryu, S., Bates, M. E., Simmons, J.A. and Riquimaroux, H. (2010). FM echolocating bats shift frequencies to avoid broadcast-echo ambiguity in clutter. *Proc. Nat. Acad. Sci. USA* **107**, 7048-7053.

### ABSTRACT

Sonar broadcasts are followed by echoes at different delays from objects at different distances. When broadcasts are emitted rapidly in cluttered surroundings, echo streams from successive broadcasts overlap and cause ambiguity in matching echoes to corresponding broadcasts. To identify reactions to ambiguity in clutter, echolocating bats that emit multiple-harmonic frequency-modulated (FM) sounds were trained to fly into a dense, extended array of obstacles (multiple rows of vertically hanging chains) while the sonar sounds the bat emitted were recorded with a miniature radio microphone carried by the bat. Flight paths were reconstructed from thermal-infrared video recordings. Successive rows of chains extended more than 6 m in depth, so each broadcast was followed by a series of echoes from multiple rows of chains that lasted up to 40 ms. Bats emitted sounds in pairs ("strobe groups") at short (20–40 ms) interpulse intervals (IPIs) alternating with longer IPIs (>50 ms). For many short IPIs, the stream of echoes from the first broadcast was still arriving when the second broadcast was emitted. This overlap caused ambiguity about matching echoes with broadcasts. Bats shifted frequencies of the first sound in each strobe group upward and the second sound downward by 3–6 kHz.

When overlap and ambiguity ceased, frequency shifts ceased also. Frequency differences were small compared with the total broadcast band, which was 75–80 kHz wide, but the harmonic structure of echoes enhances the differences in spectrograms. Bats could use time–frequency comparisons of echoes with broadcasts to assign echoes to the corresponding broadcasts and thus avoid ambiguity.

## INTRODUCTION

Echolocating big brown bats (*Eptesicus fuscus*) use sonar to guide flight and find prey in the dark (Griffin, 1958; Neuweiler, 2000). Their sonar broadcasts are frequency-modulated (FM), sweeping downward from ~110 kHz to 20 kHz in several harmonics (e.g., FM1, FM2, FM3) (Fig. 1A Inset) (Saillant et al., 2007; Surlykke and Moss, 2000). These bats change the interpulse intervals (IPIs), the initial high frequencies and terminating low frequencies of FM sweeps, the duration, and the amplitude of broadcasts according to surrounding conditions such as the distance to nearby objects (Jen and Kamada, 1982; Saillant et al., 2007; Simmons, 2005; Simmons et al., 2001; Surlykke and Moss, 2000). Each sonar broadcast impinges on objects at different distances to form a stream of echoes returning at different delays. Bats determine the distance to objects from the delay of echoes that arrive during the interval that follows each broadcast (Moss and Schnitzler, 1995; Simmons et al., 1995), and they can judge the depth of the target "scene" (Moss and Surlykke, 2001) from the echo-stream duration (ESD). The bat's auditory system registers echo delays out to 30 ms or more, equivalent to distances of at least 5 m (Simmons et al., 1996). The scene's composition in depth is represented faithfully by the time that elapses between each broadcast and the echoes that follow it,

but only if all of the echoes for one broadcast are allowed to return before the next broadcast is transmitted. If the bat emits a new broadcast before the current stream of echoes has run its course, later echoes of the first broadcast will fall inside the interval after the second broadcast and will be assigned an erroneously short delay relative to the second broadcast, causing one or more "phantom" targets to appear at close range. The intrusion of these echoes into the second broadcast's interval creates ambiguity about which broadcast really is responsible for their occurrence. When ambiguous echoes are assigned correctly to the first broadcast, their delays are perceived as relatively long, in accordance with the real scene, but when ambiguous echoes are assigned incorrectly to the second broadcast, their delays are perceived as anomalously short. The bat might treat the resulting phantom objects as obstacles that suddenly appear in its path. When maneuvering near vegetation, the bat must react swiftly to track a flying insect or to avoid a collision with the nearest branches and therefore must emit broadcasts in rapid succession (short IPIs). In contrast, to keep itself informed about the depth of the scene (the ESD) for path planning, the bat must emit broadcasts slowly enough (long IPIs) for echoes to arrive from the farthest reaches of the scene. Echoes from background objects, or clutter, can interfere with processing of echoes from targets of interest because they all are echoes of the same broadcasts and necessarily have similar waveforms. Clutter interference is exacerbated by broadcast–echo ambiguity, creating a worst-case situation—self-generated clutter resulting from the use of too-short IPIs. The obvious effectiveness of echolocation in clutter (Petrites et al., 2009; Simmons, 2005; Simmons et al., 2001; Surlykke et al., 2009) makes it desirable to learn how bats achieve this performance. When flying in dense clutter, big brown bats emit nearly all their sonar

sounds in pairs, called “strobe groups,” with shorter IPIs between members of the pairs set off by longer IPIs between one strobe group and the next (Moss and Schnitzler, 1995; Petrites et al., 2009; Surlykke et al., 2009; Surlykke and Moss, 2000). The intervals between strobe groups usually are long enough to allow reception of all echoes (IPIs longer than ESDs), in effect setting a depth-of-field for echolocation (Schnitzler et al., 2003), but the bat will experience broadcast–echo ambiguity if the shorter IPIs within strobe groups lead to overlap of echo streams from the more closely spaced broadcasts (IPIs shorter than ESDs). The key to evoking streams of strobe groups is to have clutter that is both dense, with numerous, closely spaced obstacles filling the space the bat must enter, and extended, with the array of obstacles stretching to the far end of the space. [e.g., Petrites et al., 2009 used up to 151 hanging chains.]

Assessing the bat’s reactions to broadcast–echo ambiguity and, more generally, to clutter, requires apparatus and methods to monitor changes in broadcasts without artifacts caused by the bat’s flight movements with respect to the recording microphone. Here, we combine two recently introduced experimental methods— radio telemetry of sounds from the flying bat for very stable recording of the transmitted signals (Hiryu et al., 2005; Hiryu et al., 2007; Hiryu et al., 2008) in tandem with flight tests using an extended array of obstacles during which bats emit sounds at sufficiently short IPIs to create the conditions for ambiguity (Petrites et al., 2009). The bat’s response to ambiguity caused by overlap of extended echo streams is specific: Whenever ambiguity occurs (IPIs shorter than ESDs), the bat shifts the frequencies of the FM sweep upward in the first broadcast and downward in the second broadcast by several kilohertz ($\Delta F$) in proportion to the degree of overlap (IPI minus ESD). When the bat has moved farther along its flight path,

to the point at which ambiguity no longer occurs (IPIs longer than ESDs), the bat stops making this frequency shift.

## MATERIALS AND METHODS

### Subjects

Three big brown bats (*Eptesicus fuscus*, males >4 years old) were wild-caught in Rhode Island with a permit from the state's Department of Environmental Management. They were housed in individual cages at 22–23 °C and 40– 50% relative humidity with free access to food (mealworms; *Tenebrio* larvae) and vitamin-enriched water. The day–night cycle of the room was reversed at 12-h dark/12-h light so experiments could be conducted during the daytime. Body weight was 17–20 g. Experiments complied with Principles of Animal Care, publication #86–23 (1985) of the National Institutes of Health. Procedures were approved by the Brown University Animal Care and Use Committee.

### General Experimental Procedures

The flight chamber measured 8.5 m long × 3.3 m wide × 2.4 m high. It was wrapped with copper mesh (40 threads/cm), to shield the room from external electromagnetic interference. Walls and ceiling were covered with chemically inert acoustically absorbing Sonex panels (Ilbrook Corp.), which damped background echoes. The obstacle array was made of black plastic chains (Fig. 1A) suspended vertically from the ceiling (Petrites et al., 2009). Each chain was 2.4 m long (from ceiling to floor), with oval links 7 cm long and 4 cm wide, made from plastic rings 8 mm in diameter. The

horizontal width of the chain array (12 columns of chains) was 3.2 m, and the horizontal depth of the array facing the bat (13 rows of chains) was 4.8 m. With its rectangular plan, the array provided the bat a maximum diagonal near-to-far distance of slightly more than 6 m (Fig. 1A). Adjacent chains within horizontal rows were spaced 25 cm apart (a bat's wingspan is 30–33 cm); the rows were spaced in depth at 40 cm intervals to deter bats from flying laterally between adjacent rows. For initial training, chains were removed from successive rows in the center of the array to create a flight alley 75 cm wide. Bats received a mealworm after they landed on the far wall (Petrites et al., 2009). After training, the chain array was rearranged to fill in the farther part of the alley so that during the experiments the bat flew into the enclosed space surrounded on three sides by hanging chains (Fig. 1A). The bat was released from the near end of the room in darkness, between the two video cameras, and was allowed to fly into this space before turning back (blue track in Fig. 1A).

Flights were recorded by two synchronized thermal-imaging infrared video cameras (Indigo/"Merlin" midrange type with 25-mm lenses; FLIR Systems) that could form distinct, easily tracked video images of the bat from its own body heat as it flew along the room in complete darkness. The cameras were placed 1.4 m above the floor, separated by 2 m, and located outside the copper-mesh shielding so their internal electronics did not interfere electromagnetically with the telemetry recording. Video images were recorded at 30 frames per second on the video channel of an SIR- 1000W digital instrumentation data recorder (Sony Precision Instruments). Three-dimensional coordinates of the bat's flight path were reconstructed from the stereo video recordings using Motus v2.2 motion-analysis software (Peak Performance Technologies, Inc.). The

blue curve in Fig. 1A shows one representative flight path in the chain array. The shorter (640 ms) segment of this path highlighted in red refers to the spectrograms in Fig. 1C and 1D.

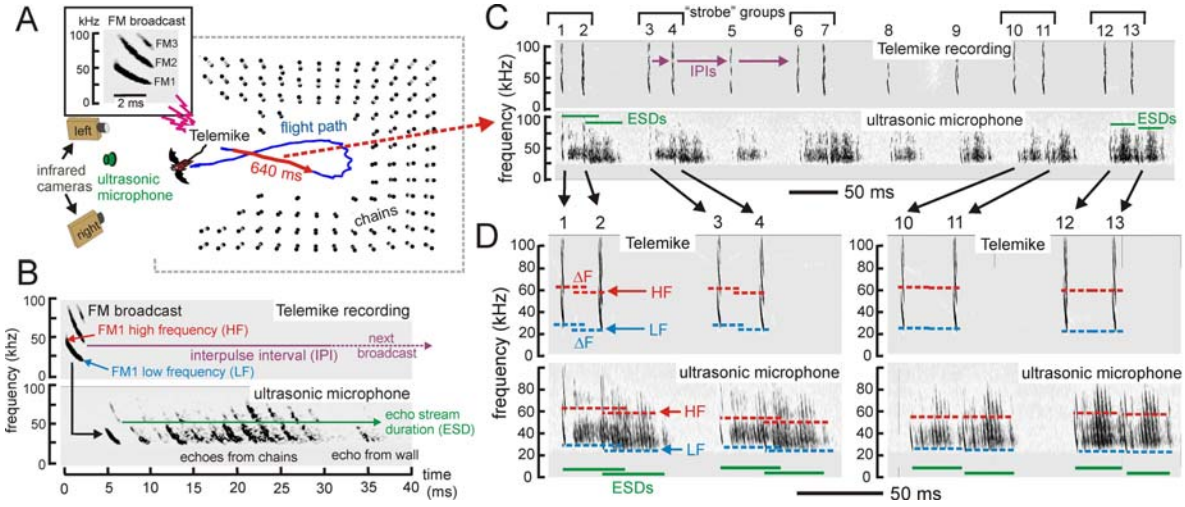


**Fig. 1.** (*A*) Perspective plan view of chain array from above, with video-reconstructed bat flight path in blue. The red section of the blue track traces the 640 ms segment related to sounds in *C* and *D*. {Inset} Spectrogram of a typical FM big brown bat broadcast during flight (harmonics are FM1, FM2, and FM3). (*B*) Spectrogram of Telemike channel (*Upper*) shows single FM broadcast. Corresponding ultrasonic microphone channel (*Lower*) shows stream of echoes from chains and wall. Labels show parameters extracted from sounds (IPI, high frequency, low frequency, ESD). (*C*) Spectrograms of broadcasts and echoes recorded during 640 ms red-marked segment of flight at Telemike (*Upper*) and ultrasonic microphone (*Lower*). IPIs show strobe groups. The ultrasonic microphone registered smears of echoes from the chains following each broadcast. (*D*) Expanded view of broadcasts 1 and 2, 3 and 4, 10 and 11, and 12 and 13 to show IPIs, ESDs, and ΔFs for strobe groups. HF, high frequency; LF, low frequency.

## Sound Recordings

The bat's sonar pulses were recorded by a custom-made telemetry microphone (Telemike) mounted on the bat's upper back and head (Hiryu et al., 2005; Hiryu et al.,

2007; Hiryu et al., 2008). It registered the bat's FM broadcasts (Fig. 1A) without Doppler shifts, amplitude changes, or spectral distortions using a small (3 mm diameter) omnidirectional Knowles FG-3329 electret microphone with response to above 100 kHz (i.e., beyond the upper limit of a bat's hearing; Koay et al., 1997). After fur was removed from the bat's upper back and head by brief application of Nair depilatory cream (Church and Dwight Co.), the Telemike was attached with double-sided tape, with its microphone pointing forward, between the bat's ears and ~1 cm above the bat's mouth. This microphone's very small diameter rendered it virtually omnidirectional over the restricted range of relative motion between the bat's open mouth and the microphone itself, so that the recordings retained their fidelity even if the bat moved its head during flight. The Telemike unit weighed <0.6 g, including the battery (light enough to be carried by bats weighing as little as 6–8 g; Hiryu et al., 2007). Big brown bats are robust (14–22 g) and exhibited no fatigue from carrying the Telemike on multiple flights. The Telemike transmitted bat-sound–modulated radio signals with a carrier frequency between 90 and 105 MHz to an FM radio antenna (AM/FM amplified antenna; Radio Shack Corp.) attached to the ceiling of the flight chamber. The received signals were demodulated using a custom-made FM receiver, amplified and high-pass filtered at 10 kHz, then digitized at a sampling rate of 384 kHz (16-bit accuracy) and recorded on the Sony data recorder. A second, stationary ANABAT II ultrasonic microphone (Titley Electronics, Ltd.) was placed 1.25 m above the floor and aimed toward the chain array behind the bat's flight path (Fig.1A).The signals from this microphone also were digitized at a sampling rate of 384 kHz and recorded on the Sony data recorder, so that the sounds recorded by both microphones were synchronized with the video recording. The

stationary ultrasonic microphone was more sensitive than the Telemike; it picked up echoes reflected back by the chains as well as the bat's broadcasts (Fig. 1 B–D; Hiryu et al., 2005). Strong echoes were reflected by all the rows of chains over a span of 20–30 ms, corresponding to the depth of the chain array from the bat's position (Fig. 1B).

**Data Analysis**

Each flight trial was digitized into a Dell Pentium-3 computer using software supplied with the data recorder (Sony Real-Time and Streamer programs). The segment containing the flight, with the sounds from the Telemike and the ultrasonic microphone, was clipped out and saved as a video file (.avi). Flight tracks of bats were reconstructed from the stereo video images using the video motion-analysis software. Acoustic characteristics of the bat's sounds were analyzed by extracting the two corresponding sound channels (Telemike and ultrasonic microphone) and displaying spectrograms using Adobe Audition v1.5 or spectrogram routines written in MatLab. IPIs, durations, amplitudes, high and low frequencies, and the frequency of the peak amplitude in FM1 were determined from the spectrograms of individual Telemike sounds (Fig. 1 C and D). Of these measures, the dimensions of FM1 (high and low frequency, frequency of peak amplitude, and duration) are the most useful parameters for describing the bat's harmonically structured signals (Fig. 2 D, E, G, and I). For each sound, the frequency of peak amplitude was determined from the spectrogram of FM1, and then high and low frequencies were determined by finding the upper and lower frequencies at which FM1 amplitude had declined by 25 dB relative to this peak amplitude. The Telemike's microphone, positioned over the bat's open mouth, picked up these features effectively

except for occasional sounds in which rf noise prevented measurement of all parameters. (These instances are the reason different numbers of strobe-group pairs were measured for high frequency and low frequency.) The ESD for each broadcast (Fig. 1B) was determined from the signals at the ultrasonic microphone using additional MatLab routines. During flights in dense arrays of chains, the bats most often emitted sonar pulses in pairs ("strobe groups;" Fig. 1C) that were closer together than the mean IPI (Petrites et al. 2009). Then, overlap of echo streams from one broadcast to the next occurred whenever the IPI was less than the ESD.

## RESULTS

### Occurrence of Broadcast–Echo Ambiguity during Flights in Clutter

Both during training, when the arrangement of chains incorporated a narrow alleyway for the bat to fly entirely through the array, and during experimental measurements, when the alleyway was partly filled in to make the bat turn back, the bats successfully negotiated the obstacles and avoided collisions. On each experimental flight, the bat carrying the Telemike flew into the open area surrounded by the chain array, circled, and then flew back out. Fig. 1A shows a plan view (from above) of a sample flight path reconstructed from stereo video images during a typical trial. Video and acoustic data were taken from a total of 30 flight sessions with three bats (12, 11, and 7 flights by different bats, respectively). The spectrogram (Fig. 1A, Inset) shows a typical multiple-harmonic FM bat sound recorded by the Telemike, with harmonics labeled FM1, FM2, and FM3. The fixed ultrasonic microphone also recorded echoes in sequence from all rows of the chain array. Fig. 1B shows the spectrogram for a broadcast signal at

the Telemike plus spectrograms for the same sound and its echoes acquired by the fixed ultrasonic microphone. Relevant acoustic features such as IPI, the high and low frequencies for estimating ΔF, and ESD are labeled in Fig. 2B. The data reported here are from flights into the array, before the bat turned back toward the cameras, so that the Telemike and the ultrasonic microphone could be used together to characterize the bat's behavior. Time intervals between successive chain echoes in Fig. 1B were about 2.3 ms, corresponding to the 40 cm spacing of successive rows of chains. Depending on the bat's position along its flight path (see track in Fig. 1A), chain echoes arrived over a span of time (ESD) ranging from ~10 to ~50 ms (mean 31.4 ms, SD 12.9) following each broadcast, culminating with an echo from the far wall (Fig.1B). The chain array effectively replicated situations involving dense, range-extended clutter, such as a bat would encounter when flying in close proximity to vegetation (Muller and Kuc, 2000; Yovel et al., 2008).

In a typical flight (Fig. 1A), the bat emitted sonar broadcasts in strobe groups with alternating short and long IPIs (Fig. 1C; see also the sawtooth curve with gray data-points in Fig. 2A). Consequently, the distribution of IPIs across flights was bimodal, with a short IPI peak at 20–40 ms and a long IPI peak at 50–80 ms. As the bat flew farther into the array (red segment of blue track in Fig.1A), ESDs shortened progressively (green horizontal bars in Fig. 1D) because fewer rows of chains remained in front of the bat. Across each flight, ESDs ranged from about 45 ms down to 20 ms (green data-points in Fig. 2A). IPIs between strobe groups were sufficiently long for each ESD to be completed before the next broadcast was emitted. In addition, some IPIs within strobe groups were slightly longer than ESDs, so broadcast–echo ambiguity still was avoided

(gray data-points in Fig. 2B). However, especially early in the flight when the depth of the array facing the bat was greatest, IPIs within strobe groups often were shorter than ESDs (e.g., sound pairs 1 and 2 and 3 and 4 in Fig. 1D). In these instances, pulse–echo ambiguity occurred (pink data-points in Fig. 2B). Later in the flight, within-strobe-group IPIs of about the same length became longer than ESDs because the depth of the array was less (e.g., sound pairs 10 and 11 and 12 and 13 in Fig. 1D). For all broadcasts in which both IPIs and ESDs could be measured (n = 1,726), 87% had IPIs longer than ESDs, and 13% had IPIs shorter than ESDs. Most instances in which IPIs were shorter than ESDs occurred within strobe groups emitted early in the flights, and they signified the presence of pulse–echo ambiguity.

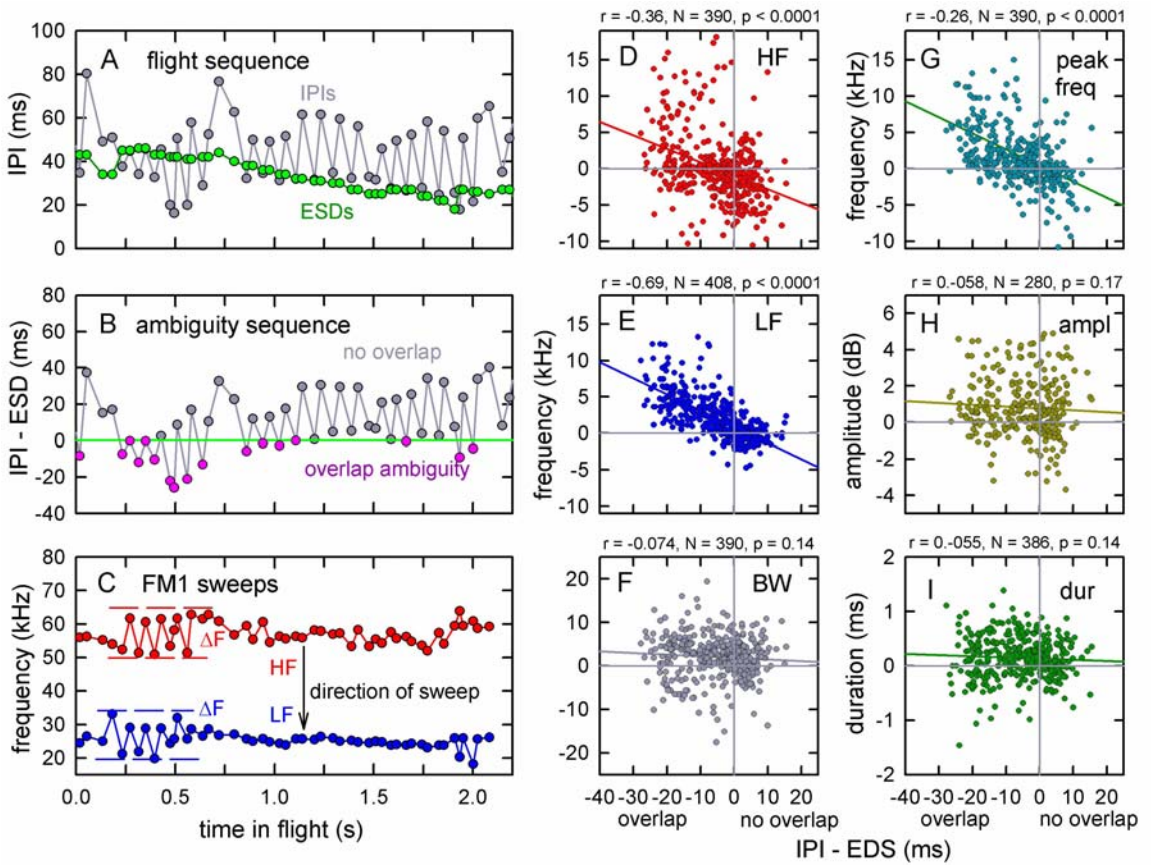


**Fig. 2.** (*A*) Plots of successive IPIs (gray) and ESDs (green) for one representative flight. (*B*) Plot showing overlap (pink data-points) or nonoverlap (gray data-points) of ESDs, with consequent pulse-echo ambiguity from one sound to the next for strobe groups (negative vertical-axis values represent overlap). (*C*) Plot showing high (HF) and low (LF) frequencies in FM1 for successive sounds from the same flight. When ESDs overlap, frequencies of the first sound are higher and frequencies of the second sound are lower (ΔF). Between the first and the second sound of each strobe group, plots show dependence of FM1 HF (*D*), FM1 LF (*E*), FM1 bandwidth (BW) (*F*), FM1 frequency at peak amplitude (*G*), relative sound amplitude (*H*), and sound duration (*I*) on the amount of ESD overlap. Plots in *D*-*I* are labeled with value of correlation coefficient (*r*), number of sound pairs (*N*), and significance of correlation (*P*).

**Frequency Shifts Within Strobe Groups**

During the measured segments of all 30 flights going into the chain array (i.e., left-to-right part of track in Fig. 1A), for n = 1,133 sounds, the mean high frequency of FM1 was 55.5 kHz (SD 5.0), and the mean low frequency was 25.4 kHz (SD 2.8). The mean bandwidth of FM1 by itself was 30.1 kHz (SD 5.5). Mean broadcast duration was 1.8 ms (SD 0.5). The critical comparisons for characterizing responses to pulse–echo ambiguity are between the first and second sounds in strobe-group pairs relative to the degree of overlap between ESDs in those strobe groups. Across all flights, 390 strobe-group pairs (780 broadcasts) were measurable for high frequency and 408 strobe-group pairs (816 broadcasts) were measurable for low frequency. Spectrograms in Fig. 1D reveal that the bat changes the frequencies of FM1 in each strobe-group pair of sounds by several kilohertz (ΔF) if their ESDs overlap (horizontal green bars for sounds 1 and 2 and for 3 and 4) but not if their ESDs are separated (sounds 10 and 11 and 12 and 13). (Presumably the occurrence of a long ESD following one or more broadcasts indicates to the bat the need for such a frequency shift on subsequent broadcasts; the data-points in Fig. 2 B and C show very prompt response when the IPI is shorter than the ESD.) The frequency of the FM1 of the first sound of the pair is raised, whereas the frequency of the second sound is lowered (Fig. 2C; high frequency, red data-points and curve; low frequency, blue data-points and curve), so that both the starting and the ending frequencies of FM1 harmonics in the two sounds diverge in frequency. Moreover, changes in frequency of FM1 are proportional to the amount of ESD overlap ($r=-0.36$ for high frequency; $r=-0.69$ for low frequency; for both, $P < 0.0001$). As would be expected, the frequency of peak amplitude in FM1 changed along with high and low frequency

(Fig. 2G), but the overall bandwidth of FM1 remained unchanged (Fig. 2F). Across bats and flights, FM1 diverged by 3–6 kHz between the first and the second sounds (y-values for regression lines in Fig. 2 D, E, and G where they intersect x-values at −25 ms overlap, which is the left-hand limit of the data distribution). FM1 slid higher or lower in frequency as a unit, carrying the higher harmonics (FM2, FM3) with it, thus altering the shape of the spectrogram beyond merely introducing ΔF to FM1 alone (Fig. 3). In contrast, neither broadcast amplitude (Fig. 2H) nor broadcast duration (Fig. 2I) changed. The overall upper frequency of the broadcasts also remained fixed at about 100–105 kHz in either FM2 or FM3, corresponding to the upper-frequency limit of the bat's hearing (Koay et al., 1997), thus capping the total sonar band, including harmonics, to frequencies ranging from 100 kHz down to each sound's low frequency (about 25 kHz).

## DISCUSSION

Our observations reveal that, when ESDs overlap and pulse–echo ambiguity occurs, the measured frequencies in successive biosonar sounds are shifted away from each other by about 3–6 kHz (Fig. 2D, E, and G), without accompanying changes in signal amplitude or duration (Fig. 2 H and I). We conclude, first, that these frequency shifts result from the bat's response, because the miniature microphone in the Telemike system protrudes forward from the Telemike circuit board on the bat's back and neck to occupy a recording position slightly forward of the external ears and just above the bat's open mouth (Hiryu et al., 2005; Hiryu et al., 2007; Hiryu et al., 2008). This location serves as a constant acoustic "vantage point" for measuring changes in FM1, so that the observed frequency differences can be attributed reasonably to the bat's vocalizations

themselves. Moreover, because both broadcast amplitude and duration remain unchanged (Fig. 2 H and I), the measured frequency shifts must indeed be frequency shifts, not artifacts of interactions that might affect threshold cutoff points used to define values for low or high frequency from spectrograms.

The ΔFs that we observed (3–6 kHz) are similar in size to frequency shifts that frequency-modulating echolocating bats make in other situations where interference is likely to occur: Conspecific bats flying in proximity shift their broadcast FM1 low frequencies away from each other, presumably to prevent interference with each other's sonar (Gillam et al., 2007; Miller and Degn, 1981; Ulanovsky et al., 2004). In target-detection experiments, big brown bats avoid the frequency of continuous jamming tones in the range of 18–32 kHz by changing the low frequencies of FM1 in their broadcasts (Bates et al., 2007). In these previous studies, observed ΔFs of FM1 low frequency range from about 1– 5 kHz (whether FM1 high frequencies change is not reported). Our second conclusion is that the observed frequency differences of several kilohertz in FM1 constitute the big brown bat's adaptive response to possible acoustic interference. To be effective, these frequency shifts must be sufficient in size for bats to segregate one broadcast and its associated echoes from other broadcasts and their echoes and from interfering constant-frequency sounds.

The upper limit of the big brown bat's effective echolocation band is determined by the upper frequency limit of recorded broadcasts at 100–110 kHz (Saillant et al., 2007; Surlykke and Moss, 2000) (100 kHz in the Telemike recordings) in conjunction with the upper limit of the bat's hearing, which is at 100 kHz (Koay et al., 1997). The lower limit of the echolocation band is determined by the low frequencies for FM1, which are around

25 kHz unless they undergo a shift (see Fig. 2 C and E). The bat's echolocation band thus spans 75–80 kHz, so that ΔFs of 3–6 kHz are only 4–8% of total bandwidth. How do such small differences allow the bat to segregate echoes arriving during overlapping ESDs so they can be associated with their correct broadcasts? After all, more than 90% of the frequencies contained in the two FM broadcasts within each strobe group are the same.

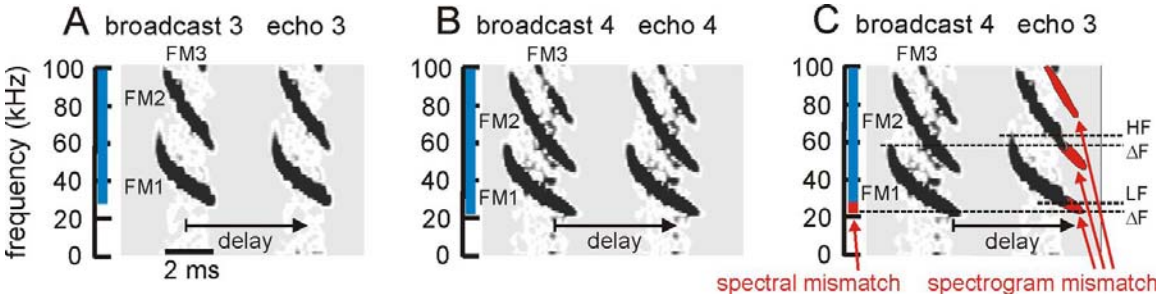


**Fig. 3.** Spectrograms for (*A*) broadcast #3 followed by idealized echo of #3, (*B*) broadcast #4 followed by idealized echo of #4, and (*C*) broadcast #4 followed by idealized echo of #3 (from Fig. 1 *C* and *D*). Echo delay is indicated by the horizontal black arrow. Vertical blue bars on frequency axis in *A*-*C* show spectrum for echo; short red bar in *C* shows one-dimensional spectral mismatch (ΔF) between broadcast #4 and echo #3. Red areas on spectrogram in *C* show two-dimensional time-frequency mismatch between the spectrogram of echo #3 and the spectrogram of broadcast #4. Spectral mismatch is about 6% (red bar vs. blue bar + red bar); because of the harmonic structure, the spectrogram mismatch is >50% (red areas vs. black areas + red areas).

Fig. 3 shows spectrograms for representative FM broadcasts and idealized echoes from a typical strobe group (broadcasts #3 and #4 in Fig. 1D). These plots display the sounds' frequency sweeps on the time–frequency plane to illustrate properties that may explain how small frequency differences between broadcasts might contribute to disambiguation of overlapping ESDs. The frequencies of FM1 (typically 25–55 kHz unless shifted by ΔF) are emitted with the widest beam (Ghose and Moss, 2003; Hartley

and Suthers, 1989), and they are the frequencies least affected by atmospheric absorption (Griffin, 1958; Lawrence and Simmons, 1982; Neuweiler, 2000), so they arrive more or less intact from the chains at the bat's ears. If IPIs are longer than ESDs, no echoes will intrude into the period about 30–35 ms after any given broadcast, which has spectrograms that differ markedly from the broadcast with respect to FM1 (Fig. 3A and 3B). In contrast, for strobe groups with IPIs shorter than ESDs, the bat's upward frequency shift in the first broadcast (broadcast #3) relative to the downward frequency shift in the second broadcast (broadcast #4) will always result in echoes of the first sound having slightly higher FM1 frequencies than echoes of the second sound (Fig. 3C). Recordings such as those illustrated in Fig. 1 C and D confirm that this generalization holds true for actual echoes from the chains. When echoes toward the end of the ESD for the first sound (e.g., #3) intrude into the early portion of the ESD for the second sound (e.g., #4), the incorrectly matched, ambiguous echoes (e.g., echo #3 following broadcast #4 in Fig. 3C) will be shifted upwards in frequency compared with the most recent broadcast and compared with the correctly matched echoes of the most recent broadcast (Fig. 3B).The 75–80 kHz spectrum of each broadcast (vertical blue bar on each frequency axis in Fig. 3) is changed only by the amount ΔF at the low-frequency end of FM1 (red segment of vertical blue bar in Fig. 3C) because the spectrum otherwise is continuous from each individual low frequency all the way up to 100 kHz. The raw spectral difference between correct and ambiguous echoes is quite small (7% in Fig. 3C). However, for ambiguous strobe-group pairs, the entire FM1 sweep (low frequency, high frequency, and peak frequency together) slides upward or downward by several kilohertz (Fig. 2 D, E, and G). This change affects the two-dimensional spectrograms more dramatically than the one-

dimensional spectrum because the higher harmonics are linked to FM1 by integer multiples (2× for FM2, 3× for FM3). On the time–frequency plane of Fig. 3C, the harmonic sweeps in echoes of the upward-shifted first broadcast become separated from the harmonic sweeps in echoes of the downward-shifted second broadcast to create large areas in which the spectrograms of the echoes do not match (red areas in right-hand plot of Fig. 3C are more than 50% of total areas).The key is use of spectrograms to register on the time–frequency surface the consequences of small frequency shifts such as those measured in FM1 (Fig. 2 D, E, and G). These shifts are passed to the higher harmonics by the integer relations of their frequencies, magnifying the effective difference between matched echoes (#3 with #3, #4 with #4 in Fig. 3A and B) and mismatched echoes at FM2 and FM3 (#4 with #3 in Fig. 3C).

Multiple studies have shown that various sites in the auditory pathway (e.g., inferior colliculus, auditory cortex) of frequency-modulating bats (Bodenhamer and Pollak, 1981; Casseday and Covey, 1996; Pollak et al., 1977; Suga et al., 1990), including big brown bats (Ferragamo et al., 1998; Ma and Suga, 2008; Sanderson and Simmons, 2000; Sanderson and Simmons, 2002; Sanderson and Simmons, 2005), register the brief FM sweeps in biosonar broadcasts and echoes on a frequency-by-frequency basis using responses of neurons tuned to individual frequencies distributed across the 15–100 kHz band. When big brown bats are stimulated by multiple-harmonic FM sounds of 1- to 3-ms duration, comparable to those emitted and received during flights through the chains, each neuron produces approximately one spike that marks the time-of-occurrence of that cell's best excitatory frequency along the sweep (Bodenhamer and Pollak, 1981; Casseday and Covey, 1996; Ferragamo et al., 1998; Ma and Suga, 2008;

Pollak et al., 1977; Sanderson and Simmons, 2000; Sanderson and Simmons, 2002; Sanderson and Simmons, 2005; Suga et al., 1990). For each broadcast or each echo, individual sweeps of FM1 or FM2 evoke a series of on-responses occurring across a population of neurons having different best excitatory frequencies. For any one sound, these spikes are distributed across time (onset-spike latency) in neurons distributed across (best) frequency, to form the time–frequency plane for a kind of neuronal spectrogram (Simmons et al., 1996; Suga et al., 1990). The use of FM signals and the pattern of neuronal responses have thus prompted speculation that bats not only might use a frequency-by-frequency representation but also might exploit the advantages of true time– frequency spectrograms (Bodenhamer and Pollak, 1981; Griffin, 1958; Neuweiler, 2000; Simmons et al., 1996; Suga et al., 1990). Further investigation of how echolocating bats resist clutter interference and segregate echoes in conditions of pulse–echo ambiguity will focus on the perceptual consequences of removal or displacement of segments of FM sweeps in echoes, such as those illustrated by the red areas in Fig. 3C. Recently, big brown bats were trained to discriminate differences in the delay of electronically generated echoes between "normal" echoes containing FM1 and FM2 at the same delay (positive stimuli at fixed delay of 3.2 ms) and modified echoes with FM2 delivered 300 μs later than FM1 ("split-harmonic" echoes as negative stimuli with FM1 at 3.2 ms and FM2 at 3.5 ms at nominal zero delay difference, with additional delay of up to 1 ms to negative stimuli for descending method-of-limits psychophysics; Stamper et al., 2008). The bat's delay acuity is disrupted radically: Thresholds for detecting delay differences change from ~50 μs (as in normal echo delay-discrimination tests) to ~800 μs in delay-discrimination tests with split-harmonic echoes (Stamper et al., 2008). This

result suggests that bats may experience blur in the perception of delay for mismatched echoes in ambiguous conditions to prevent the worst case of clutter interference.

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CHAPTER 4

# Effects of filtering of harmonics from biosonar echoes on delay acuity by big brown bats (*Eptesicus fuscus*)

Bates, M. E. and Simmons, J. A. (2010). Effects of filtering of harmonics from biosonar echoes on delay acuity by big brown bats (*Eptesicus fuscus*). *J. Acoust. Soc. Am.* **128**, 936-946.

## ABSTRACT

Big brown bats emit frequency-modulated (FM) biosonar sounds containing two principal harmonics (FM1 ~55-22 kHz; FM2 ~105-45 kHz). To examine the role of harmonics, they were selectively filtered from stimuli in electronic echo delay-discrimination experiments. Positive stimuli were delayed by 3.16 ms (55 cm simulated target range); negative stimuli were delayed by 3.96 ms (68 cm). This large 800 μs delay difference (nearly 14 cm) was easily discriminated for echoes containing equal-strength FM1 and FM2. Performance gradually decreased as highpass filters removed progressively larger segments from FM1. For echoes with FM2 alone, performance collapsed to chance, but performance remained good for lowpass echoes containing FM1 alone. Attenuation of FM2 by 3 dB relative to FM1 also decreased performance, but shortening electronic delay of the attenuated FM2 by 48 μs counteracted amplitude-latency trading and restored performance. Bats require the auditory representations of FM1 and FM2 to be in temporal register for high delay acuity. Misalignment of neuronal responses degrades acuity, but outright removal of FM2, leaving only FM1, causes little

loss of acuity. Functional asymmetry of harmonics reflects lowpass filter effects from beaming and atmospheric propagation, which leaves FM1 intact. It may cooperate with latency shifts to aid in suppression of clutter.

## INTRODUCTION

Big brown bats (*Eptesicus fuscus*) emit trains of wideband frequency-modulated (FM) biosonar signals at frequencies of about 22–105 kHz that contain multiple harmonics (Saillant et al., 2007; Surlykke and Moss, 2000). They use echoes of these sounds to detect and intercept prey, avoid obstacles, and navigate through spatially complex surroundings such as vegetation (Griffin, 1958; Neuweiler, 2000). The two most prominent harmonics in the broadcasts are the first (FM1), which typically sweeps downward from 55 to 22 kHz, and the second (FM2), which sweeps downward from 105 to 45 kHz. Although big brown bats often capture flying insect prey, they also are observed to forage near clutter, such as dense vegetation, and to glean insects from the ground or other surfaces (Simmons, 2005; Simmons et al., 2001). Flying near vegetation or the ground exposes the bat to clutter that could obscure the presence of objects of interest, such as insects to be pursued or obstacles to be avoided. Bats would encounter difficulty in detecting echoes from such objects if these are masked by the more intense echoes from background clutter (Schnitzler et al., 2003). Several FM bat species nevertheless are able to detect and capture prey located close to clutter (Jensen et al., 2001; Moss et al., 2006; Siemers and Schnitzler, 2000). Furthermore, laboratory tests reveal that bats can fly along narrow, turning paths surrounded by dense, deeply distributed clutter without colliding into obstacles (Petrites et al., 2009). When flying in

the densest clutter conditions, the bats alter the pattern of their broadcasts to shorten the intervals between successive sounds, often so much so that the stream of echoes returning from one sound is not finished before the next sound is broadcast and its stream of echoes begins to arrive (Petrites et al., 2009).

Overlap of echo streams for successive broadcasts results in ambiguity about which broadcast is responsible for which echoes. *Pulse-echo ambiguity* is a kind of self-generated clutter interference that places a premium on recognizing successive broadcasts as sufficiently different from one another that their echoes can be recognized as different, too. Using a miniature radio microphone carried by the bat to record subtle changes in biosonar broadcasts without artifacts related to Doppler shifts and directional beaming, big brown bats were observed to adapt to ambiguity-related clutter by shifting the frequencies of adjacent FM broadcasts away from each other by several kilohertz (Hiryu et al., 2010). Crucially, these frequency shifts only occurred when echo streams overlapped to create pulse-echo ambiguity. Although ambiguity-induced frequency shifts amount to only a few percent of the total bandwidth in broadcast spectra, in spectrograms of the sounds, up to about half of the time-frequency surface occupied by the harmonic sweeps is redistributed, making the sounds easily distinguishable. This finding suggests that bats may be very sensitive to small changes in the relative positions of different frequencies in the FM sweeps of echoes on the time-frequency plane. In effect, bats might use auditory "fingerprints" for recognizing echoes and sorting them to their corresponding broadcasts.

To assess this possibility, an experimental technique has been developed that segregates the two principal harmonics in the sonar sounds of big brown bats (FM1 and

FM2) into different channels of an electronic target simulator so that the effects of misaligning the harmonics in time could be measured. When FM2 is delayed 300 μs relative to FM1 in such a "split-harmonic" echo delay-discrimination task, the echo delay acuity of about 50 μs normally obtained in two-choice discrimination tests deteriorates extraordinarily to about 1000 μs (Stamper et al., 2009). For all practical purposes, the 300 μs offset of harmonics caused the bat's delay acuity to collapse completely.

The experiments described here were carried out to determine whether selective removal of frequencies in FM1 or FM2 affects bats' delay acuity as drastically as the 300 μs delay offset of the harmonics. For example, does slight attenuation or complete removal of FM2 cause the bat's delay acuity to become as poor as when FM2 is delayed by 300 μs more than FM1? What happens when the spectrum of FM1 is truncated or entirely removed? The split-harmonic target simulator is used to create differential changes in the amplitude or spectrum of FM1 and FM2 in echoes, allowing us to measure the effects of these acoustic manipulations on the bat's delay discrimination performance. Bats were trained to choose echoes arriving at a delay of 3.16 ms and not choose echoes arriving at a delay of 3.96 ms. In ordinary conditions, bats easily perform this 800 μs difference task with only about 5% errors. In the 300 μs split-harmonic experiment, the bat's acuity deteriorates so much that performance fell to about 16% errors, a very significant loss even though the difference is more than twice the bat's 350 μs integration time for echo detection (Simmons, 1989).

The 300 μs offset of FM2 in the split-harmonic experiment is manifested as misalignment of the harmonics on the spectrogram's time-frequency plane, not as a change in the amplitude spectrum as such. Through amplitude-latency trading (the

dependence of neuronal response latencies on stimulus amplitude; Bodenhamer and Pollak, 1981; Burkard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990), changes in the amplitude of harmonics are transposed into changes in the timing of the auditory representation of the harmonics. Amplitude-latency trading thus creates time shifts between harmonics internal to the bat. The possibility that bats recognize echoes strictly from the timing of the harmonics is explicitly tested by exploiting amplitude-latency trading—inducing a latency shift by reducing the amplitude of FM2 and then compensating for this shift by changing the delay of FM2.

In the present experiments, the bats' performance on the normally easily-discriminated 800 μs delay difference was used as an index for the effects of manipulating the harmonics in echoes. These new experiments are presented here in the order of their design and conduct (i.e., Experiments 1, 2, 3, and 4) because several of the experiments were based on results obtained from earlier conditions. Together, the results of these experiments indicate that harmonics interact with beamforming and atmospheric effects to contribute to perception of echo delay.

## MATERIALS AND METHODS

### Subjects

Four adult big brown bats (Chris, Buddy, Vlad and Marina; three males and one female) were trained to discriminate between electronically produced echoes that differed in delay by 800 μs. The bats were housed individually in a temperature and humidity controlled room on a 12:12 reverse light: dark cycle. They were given vitamin-enriched (Poly-Vi-Sol) water *ad libitum* and fed mealworms (*Tenebrio molitor* larvae) daily. All

subjects weighed between 14.5 and 15.5 g. Animal care procedures were consistent with guidelines established by the National Institute of Health and were approved by Brown University Animal Care and Use Committee.

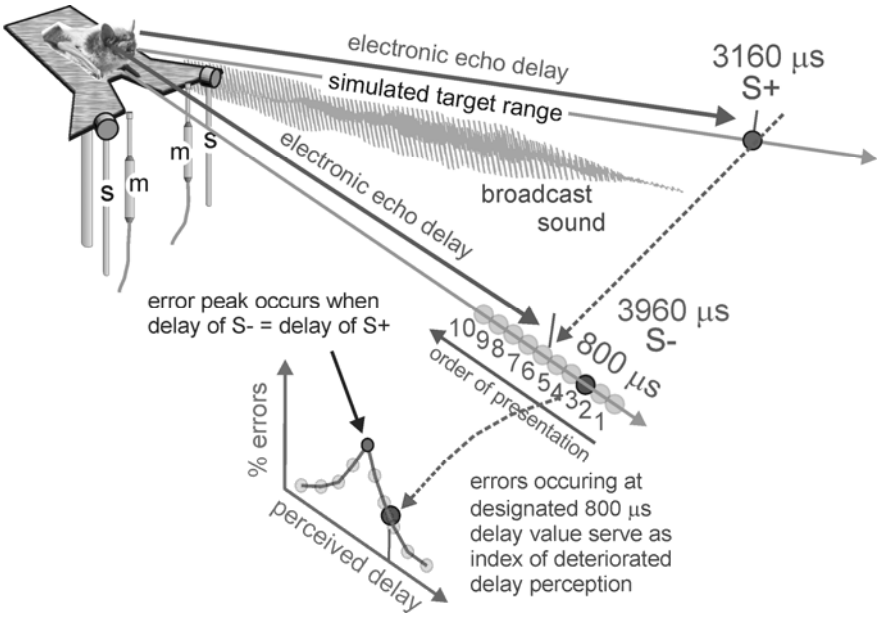


**Fig. 1.** Diagram of two-choice electronic echo delay-discrimination set-up with bat on Y-platform: Bat's broadcast sounds are picked up by microphones (m), delayed and filtered electronically, and returned as echoes of virtual targets from loudspeakers (s). Delay of S+ is fixed at 3160 μs, which simulates a target range of 54.5 cm. Delay of S- is fixed 800 μs later, at 3960 μs, which simulates a target range of 68.3 cm. The 800 μs difference between S+ and S- corresponds to the index point described in Fig. 2. For the present experiments, S+ and S- undergo various filtering conditions to assess effects on performance at 800 μs index point. Idealized percent error performance curve illustrates relation between peak in error curve when S- matches delay of S+ and the still-elevated performance at 800 μs.

**Psychophysical Test**

Figure 1 illustrates the two-alternative forced-choice task on which the bats were trained. The experiment was run in a 4 m × 3 m × 2.5 m room with panels of sound

absorbent foam (Sonex, Illbrook, Inc.) lining the floor and walls. Light levels were kept dim for training and experimental trials. The equipment for producing the electronic echo stimuli was located in an adjacent room.

The bat's task was to discriminate between the positive stimulus (rewarded echoes; S+) at a delay of 3160 μs and the negative stimulus (unrewarded echoes; S-) at a delay of 3960 μs. Each bat was trained to sit at the base of an elevated Y-platform and broadcast its echolocation sounds toward two ultrasonic microphones (Bruel & Kjaer Model 4138 "1/8-inch" condenser microphones), one located on each end of the platform. As shown in Fig. 1, microphones were mounted 20 cm away and separated by 40°. Echolocation sounds emitted by the bat were picked up by the microphones, filtered and delayed electronically and then delivered back to the bat from small electrostatic loudspeakers 15 mm in diameter (RCA, Model 112343). Speakers were mounted next to the microphones at the ends of both arms of the platform, 20 cm away from the bat and 50° apart (Fig. 1). The bat was rewarded with a piece of mealworm for walking down the arm of the Y-platform corresponding to the loudspeaker that delivered the positive stimulus, which was presented on either the left or right side in a pseudorandomized Gellerman sequence (Gellerman, 1933). If the bat made an incorrect response, a broadband sound was made to signal to the bat that it made an error. All trials were run using a double-blind procedure. Two experimenters were present while bats were run, a trainer who handled the bat and was blind to the position of the correct choice and a recorder who controlled which loudspeakers generated the stimuli and recorded the bat's response. The recorder observed the bat using a black and white CCD video camera (Supercircuits, Inc., Type 15-CB22–1) mounted on the ceiling above the Y-platform.

Illumination for the camera was provided by two infrared LED panels (Supercircuits, Inc.) located on either side of the video camera. The recorder was able to monitor the bat's performance on a Sony digital 8-mm video walkman recorder located behind the trainer and the Y-platform.

Once the bat was able to perform the task for normal echoes (baseline, full-band echo condition) at better than 90% correct responses, sets of 50 experimental trials were conducted each day for three days for every condition. Each bat completed 150 trials over three days for each stimulus condition, for which the percentage of correct responses was accumulated.

**Electronic Stimuli**

The two-channel electronic target simulator system has been described fully elsewhere; no changes were made for the new experiments described here (see Fig. 4, Stamper et al., 2009). From bat sound to echo, the total gain of this system was approximately -35 dB for S+ and -40 dB for S-. The 5 dB attenuation of S- relative to S+ approximately compensates for the 5 dB increased sensitivity of the bat for echoes at the longer delay of 3960 μs compared to the shorter delay of 3160 μs (Kick and Simmons, 1984). All echoes were highpass (HP) filtered at 20 kHz and lowpass (LP) filtered at 90 kHz to set the boundaries of the echo band and remove background noise. Additional highpass or lowpass filtering used to restrict the frequency band for echoes serving as S+ or S- was introduced using filters already present in the simulator system. All values of filter settings and attenuations relative to a system gain of -35 dB are given in Table I.

| Exp. | Stimulus condition | S+ filter settings | S+ attenuation (dB) | S- filter settings | S- attenuation (dB) |
|---|---|---|---|---|---|
| 1 | FM1 and FM2 (baseline) | 20 kHz HP<br>90 kHz LP | -15 | 20 kHz HP<br>90 kHz LP | -20 |
| 1 | FM1 only | 20 kHz HP<br>44 kHz LP | -15 | 20 kHz HP<br>44 kHz LP | -20 |
| 1 | 28 kHz HP | 28 kHz HP<br>90 kHz LP | -15 | 28 kHz HP<br>90 kHz LP | -20 |
| 1 | 32 kHz HP | 32 kHz HP<br>90 kHz LP | -15 | 32 kHz HP<br>90 kHz LP | -20 |
| 1 | FM2 only | 66 kHz HP<br>90 kHz LP | -15 | 66 kHz HP<br>90 kHz LP | -20 |
| 2 | FM1, 0 μs | 20 kHz HP<br>44 kHz LP | -15 | 20 kHz HP<br>44 kHz LP | -20 |
| | FM2, 0 μs | 66 kHz HP<br>90 kHz LP | -15 | 66 kHz HP<br>90 kHz LP | |
| 2 | FM1, 0 μs | 20 kHz HP<br>44 kHz LP | -15 | 20 kHz HP<br>44 kHz LP | -20 |
| | FM2, 300 μs | 66 kHz HP<br>90 kHz LP | -15 | 66 kHz HP<br>90 kHz LP | |
| 3 | FM2 only (S+) | 66 kHz HP<br>90 kHz LP | -15 | 20 kHz HP<br>90 kHz LP | -20 |
| 4 | FM2 -3 dB (S+) | 20 kHz HP<br>90 kHz LP | FM1: -15<br>FM2: -12 | 20 kHz HP<br>90 kHz LP | -20 |
| 4 | FM2 -3 dB – 48 μs (S+) | 20 kHz HP<br>90 kHz LP | FM1: -15<br>FM2: -12 | 20 kHz HP<br>90 kHz LP | -20 |

**Table 1.** Electronic filter settings and attenuation of stimuli for four experiments.

A series of four different experiments were conducted using specific values of echo delay and filter settings as described below. However, the experiments were not carried out independently of each other; stimulus settings used in later experiments were chosen to explore the significance of results found in earlier experiments. Consequently, it is necessary to describe the stimuli in the order they were used and the results in the order they were obtained. For each experiment, spectrograms are used to illustrate the stimuli, accompanied by graphs showing the bats' performance (percent errors in two-choice task) on these stimuli. As a complication, S+ and S- were the same in some experiments but different in others. To facilitate presentation of the experiments,

spectrograms and results are combined into one figure for each experiment. All four bats completed the entire series of experiments. Their performances are plotted in Figs. 2B, 3B, 4B, and 5B as percent errors with 0% being perfect performance and 50% being chance performance.

## EXPERIMENT 1: EFFECTS OF REMOVING HARMONICS ON DELAY ACUITY

### Procedure

Figure 2A shows spectrograms and electronic filter settings for the five stimulus conditions used for Experiment 1. The first condition illustrated by the spectrograms is the baseline, with full-band echoes (20–90 kHz), in which S+ is presented at 3160 μs and S- is presented at 3960 μs, with both FM1 and FM2 intact and arriving simultaneously. The next three spectrograms for S+ and S- (28–90 kHz; 32–90 kHz; 66–90 kHz) illustrate a progression of three stimulus conditions in which progressively larger segments of the lower-frequency end of FM1 are removed. At the end of this series, FM1 is removed entirely and the stimuli contain only FM2. The last spectrogram in Fig. 2A shows the FM1 only condition (20–44 kHz), in which the sound was lowpass filtered to selectively remove FM2, leaving only FM1. For all of the conditions illustrated in Fig. 2A, the same filtering was applied to both S+ and S-. Thus, the bat would receive echoes filtered in the same manner from both arms of the platform. For all of these conditions in Experiment 1, S+ and S- differed only in delay.

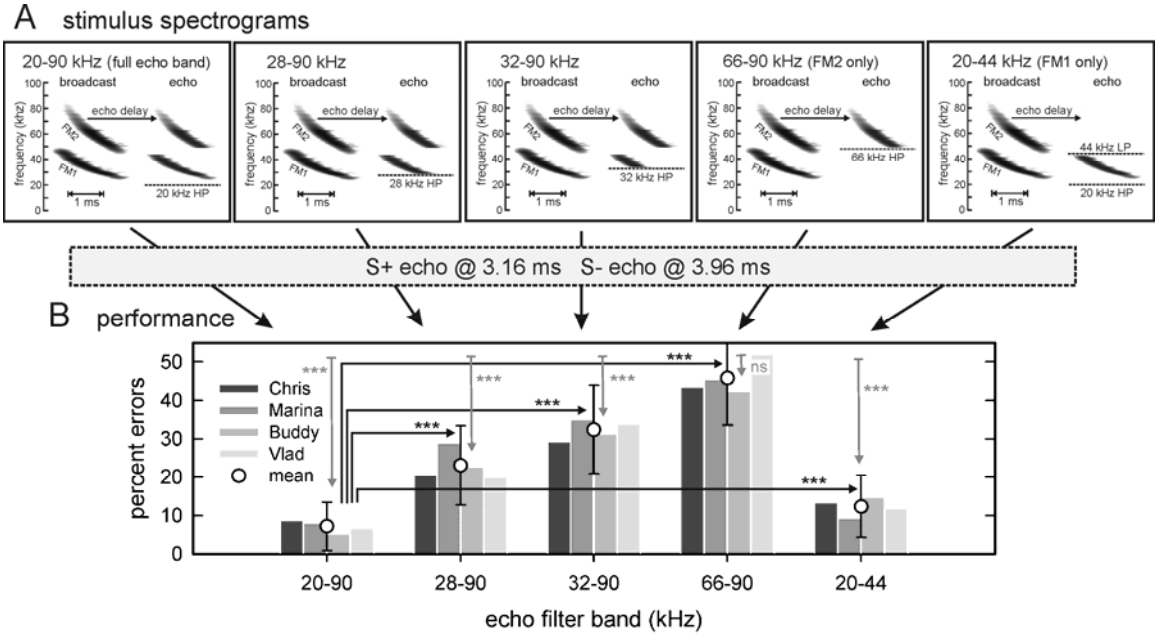


**Fig. 2.** Experiment 1. Effects of removing harmonics on delay acuity: (*A*) Spectrograms for stimuli used as echoes of a representative bat broadcast containing FM1 and FM2. Highpass filtering restricts the echo band to 20-90, 28-90, 32-90, or 66-90 kHz, which removes progressively larger segments from the low-frequency end of FM1. Lowpass filtering to 20-44 kHz completely removes FM2, leaving FM1 mostly intact. Filters have roll-off of 115 dB/octave to sharply cut out the unwanted part of the echo spectrum. Both S+ and S- are filtered as indicated and then delivered with the index delay difference of 800 μs (Fig. 3). (*B*) Bar graph showing performance on delay-discrimination tasks (percent errors) for four bats (150 trials/bat) with mean performance ± 1 SD (circles). Removal of FM1 in small stages causes progressive, significant loss of delay acuity (more errors) for 800 μs difference between S+ and S- (*** = $P<0.001$), culminating in chance performance (ns = $P>0.05$) for echoes containing only FM2. Performance recovers but still is not completely normal when echoes contain only FM1.

## Results

The bar-graph in Fig. 2B shows the performance of the four bats on the stimulus conditions of Experiment 1 (Fig. 2A). Mean performance ±1 SD is plotted over the vertical bars for the individual bats (white circles with vertical error lines). The bats' performance was uniformly good (few errors) for the baseline, full-band condition (indicated as 20–90 on horizontal kHz axis). On average, the bats made only 6% errors,

which was significantly better than chance (4 bats × 150 trials/ bat; N=600, P<0.001). (This and all subsequent statistics report probabilities from exact binomial two-tailed tests.)

Highpass filtering that removed increasingly larger portions from the low-frequency end of FM1 caused progressive losses in delay acuity (more errors) relative to the baseline, full-band condition. In the 28–90 kHz filter condition (Fig. 2B), mean percent errors was 20.9%. This was significantly different from both the baseline condition (P<0.001), and from chance (50%) performance (P<0.001). Mean percent errors in the 32–90 kHz filter condition was 30%, which differed significantly from both the baseline, full-band condition (P<0.001) and from chance performance (P<0.001). For echoes containing only FM2 in the 66–90 kHz filter condition, performance fell to an average of 45.8% errors. This was significantly worse than baseline performance (P <0.001), but it was not significantly different than chance (P=0.121).

Finally, in the 20–44 kHz filter condition, which delivered FM1 alone, the bats made an average of 12.3% errors, which differed significantly from both baseline, full-band performance (P<0.001), and from chance performance (P<0.001).

## EXPERIMENT 2: RELATIVE SALIENCE OF HARMONICS

### Procedure

The results from Experiment 1 reveal effectively chance performance (45.8% errors) for discrimination of the index 800 μs difference in echo delay if echoes contain only FM2 (66–90 kHz filtering), but good performance for echoes containing only FM1 (20–44 kHz filtering). Experiment 2 was conducted to determine whether the chance

performance for FM2 reflects poor delay acuity as such, or whether big brown bats do not treat stimuli containing just FM2 as “echoes” at all. Echoes containing FM1 alone would occur naturally for off-axis or far away targets, but echoes containing just FM2 are not normally experienced by bats. Experiment 2 presented bats with echoes containing only FM2 as S+ (66–90 kHz filter condition), and baseline, full-band echoes (20–90 kHz filter condition) as S-. Figure 3A shows the spectrograms for these stimuli. By giving the bat a choice between FM2 alone for S+ at a shorter, normally easily discriminated delay of 3160 μs and full-band echoes containing both FM1 and FM2 for S- at a much longer delay of 3960 μs, the possibility that the bats might reject FM2 can be assessed. Because the intent of Experiment 2, in conjunction with several of the conditions from Experiment 1, is to identify the relative roles of the harmonics in echoes, Fig. 3A shows spectrograms for full-band echoes (20–90 kHz) containing both FM1 and FM2, along with echoes containing only FM1(20–44 kHz) or only FM2 (66–90 kHz).

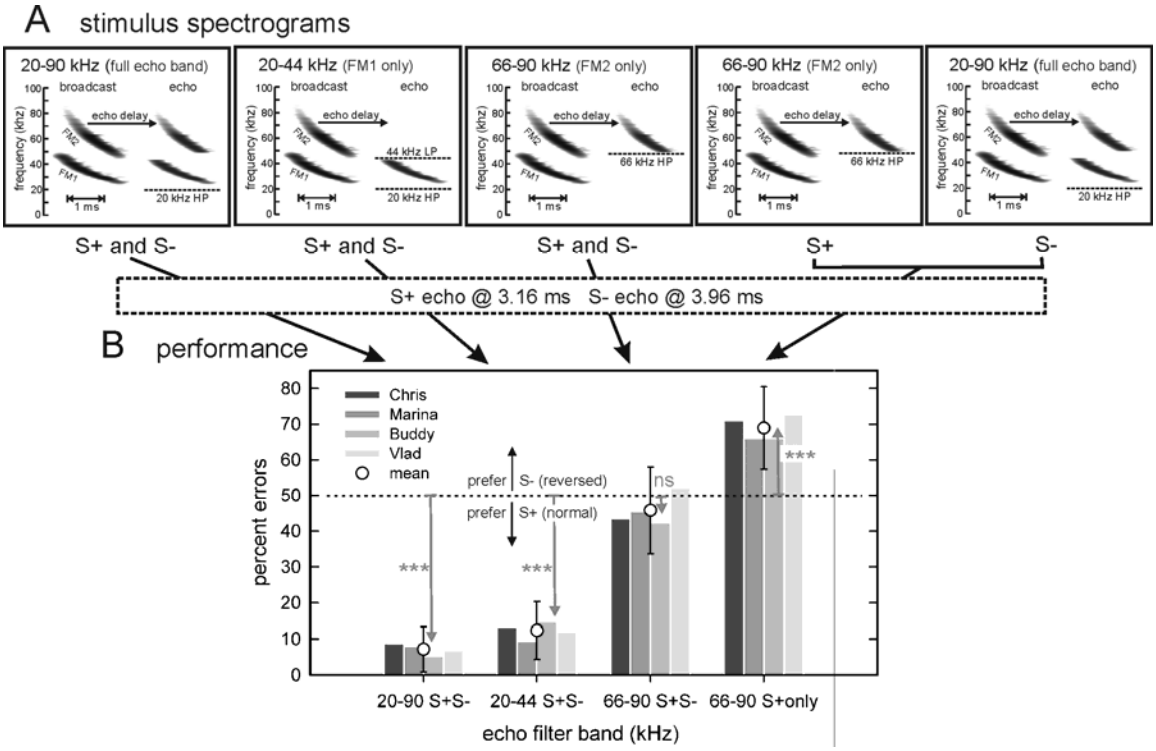


**Fig. 3.** Experiment 2. Relative salience of harmonics: (*A*) Spectrograms for representative stimuli containing only FM1 or only FM2 for S+ and S-. Full-band, normal echoes are shown at left. At right, S+ contains only FM2 while S- contains FM1 and FM2 (full band). (*B*) Bar graph showing performance on delay-discrimination tasks (percent errors) for four bats with mean performance ± 1 SD. As already shown in Fig. 2, performance on 800 µs delay difference is good (*** = P<0.001) if echoes contain only FM1 but chance (ns = P>0.05) if echoes contain only FM2. When offered a choice between S- containing FM1 and FM2 versus S+ containing FM2 alone, bats prefer S- even though it is delivered 800 µs later than S+ (*** = P<0.001).

**Results**

Figure 3B plots the individual performance of the four bats, along with the mean ±1 SD, on the several conditions with FM1 or FM2 side-by-side for comparison. The horizontal dotted line at 50% errors indicates performance at chance levels. Performance below this line, toward 0% errors, indicates that the bats prefer S+ (rewarded choice); performance above this line, toward 100% errors, indicates that the bats prefer S- over S+

(reversed preference). As previously shown in Fig. 2B, the bats' performance discriminating the 800 μs index delay difference is significantly better than chance for the baseline full-band (20–90 kHz) condition ($P<0.001$), and for the FM1 only (20–44 kHz) condition ($P<0.001$). Performance is at chance for the FM2 only (66–90 kHz) condition ($P>0.05$). The new condition for Experiment 2 examines whether this inability to select S+ reveals poor delay acuity for FM2 by itself, so that the 800 μs difference is not perceptible, or whether FM2 is simply not considered to be an echo by the bat. When given echoes containing only FM2 as S+ and full-band echoes as S-, the bats' performance *reverses* to become better than chance, but for S-, not S+ ($P<0.001$). This reversal of performance when bats are presented with echoes containing only FM2 at a much earlier delay than echoes containing FM1 and FM2 suggests that sounds containing only FM2 are not accepted as echoes, so the bats choose instead the normal, full-band echoes.

## EXPERIMENT 3: EFFECTS OF HARMONIC-SPLIT FILTERING ON DELAY ACUITY

### Rationale

Responses evoked in individual neurons of the big brown bat's inferior colliculus and auditory cortex by short duration FM sounds such as the signal illustrated in the spectrograms of Figs. 2A and 3A consist, on average, of a single action potential at a well-defined latency for each presentation of the stimulus (Sanderson and Simmons, 2000, 2002, 2005; Simmons et al., 1998). These neurons are tuned to a well-defined best frequency in the bat's 15–100 kHz echolocation band, but they respond equally well over

a wide range of stimulus amplitudes. That is, they exhibit poor tuning to specific stimulus amplitudes. However, response latencies to FM stimuli are exquisitely sensitive to amplitude changes, shifting later by about 16 μs for each decibel of decrease in amplitude (Bodenhamer and Pollak, 1981; Burkard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990). Changing the amplitude of FM1 relative to FM2 thus affects the latencies of responses evoked by individual frequencies in the FM sweep with far greater precision than it affects the probability of a response actually occurring. From Experiment 1, bats discriminate the 800 μs index delay difference progressively worse for echoes that have increasingly large segments of FM1 removed by filters (Fig. 2B). Does the change in FM1 amplitude, which leads to lengthening of the latencies of responses to FM1, disrupt the normal simultaneity, or temporal coherence, of neural responses to FM1 and FM2? Does this loss of temporal coherence within the bat's auditory representation then cause the poor performance in the 800 μs delay discrimination tests (Fig. 2B)?

The previous split-harmonic experiment, in which echoes were delivered with FM2 at a 300 μs later delay than FM1, revealed a surprising collapse in the sharpness of the big brown bat's delay image, which provided the rationale for using a large delay difference of 800 μs for the present experiments. Although this first split-harmonic experiment separated FM2 from FM1 by fully 300 μs, the split harmonic procedure itself provides a means for adjusting the delays of FM1 and FM2 independently in much smaller steps, too. If amplitude-latency trading disrupts the temporal coherence of the harmonic representations inside the bat's auditory system when the strength of one harmonic is changed relative to the other (trading ratio is −16 μs /dB), thus causing delay acuity to collapse, then shifting the *delay* of the changed harmonic by an equal but

opposite amount in time should restore coherence and remove the poor performance in the 800 μs delay discrimination task. Because this test involves measuring whether the bats make more errors or less errors in different stimulus conditions, it is important to know whether the split-harmonic filtering arrangement itself can cause some loss in delay acuity.

**Procedure**

Experiment 3 was designed to determine whether introducing the split-harmonic filter settings (20–44 kHz to isolate FM1; 66–90 kHz to isolate FM2) worsens the performance of the bats on the 800 μs delay-discrimination task by such a large amount that it would obscure any further worsening caused by changing the amplitude of one harmonic relative to the other, or by adjusting the delay of the harmonics to compensate for amplitude-latency trading. Figure 4A shows spectrograms for three stimulus conditions—on left, baseline, full-band echoes for S+ and S- (from Fig. 2A), in middle, split-harmonic conditions (20–44 kHz for FM1; 66–90 kHz for FM2) with the same delay for FM1 and FM2 (given as 0 μs relative to the total delay of 3160 μs for S + and 3960 μs for S-) or with the previously-tested 300 μs offset of FM2 relative to FM1. On right, S- consists of split-harmonic echoes with the same delay for FM1 and FM2.

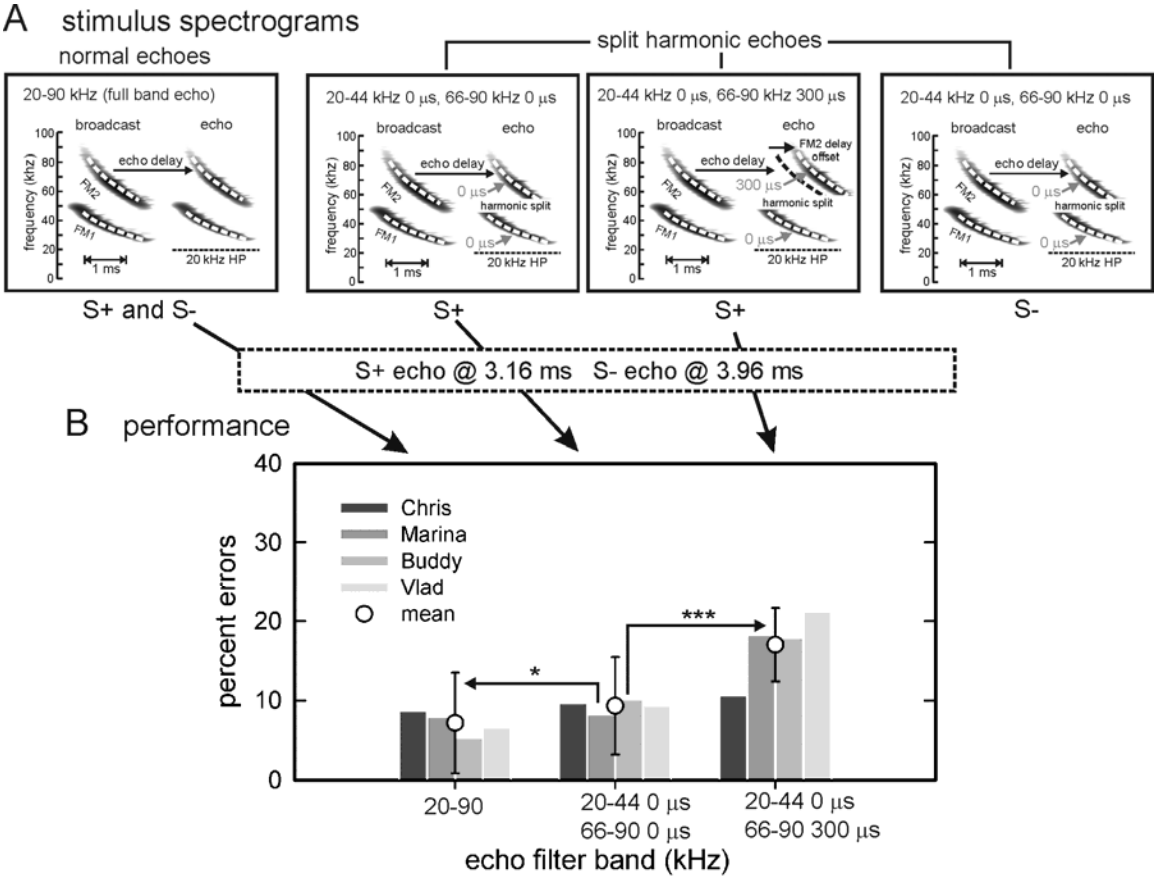


**Fig. 4.** Experiment 3. Effects of harmonic-split filtering on delay acuity: (*A*) Spectrograms for representative stimuli testing the effect of introducing the harmonic-split filters on the 800-µs index delay discrimination task. Full-band, normal echoes with no harmonic split for S+ and S- are shown at left (note slight overlap of harmonics at 50-55 kHz). Next, to split the harmonics into two parallel electronic channels, echoes for both S+ and S- are filtered at 20-44 kHz to remove FM2 and 66-90 kHz to remove FM1. The isolated harmonics are delayed and amplified differently, then recombined (added) to create split-harmonic echoes that now have a narrow spectral gap eliminating frequencies around 55 kHz where FM1 and FM2 overlap. S+ echoes have either the harmonic split alone (FM1 and FM2 both at "0 µs") or the harmonic split plus 300-µs offset of FM2 relative to FM1 (FM1 "0 µs," FM2 at "300 µs"). S- echoes have the harmonic split alone (FM1 and FM2 at "0 µs"). *(B)* Bar graph showing performance on delay-discrimination tasks (percent errors) for 4 bats (150 trials/bat, with mean performance ±1 SD shown by circles). Introduction of harmonic-split filters with no other change causes small, significant decrease in acuity (more errors) compared to full-band echoes (* = $P < 0.05$). Addition of 300-µs offset to FM2 relative to FM1 causes large decline in acuity beyond what occurs for split-harmonic condition with no delay difference (*** = $P < 0.001$).

**Results**

Figure 4B shows the performance of the four bats, and the mean performance ±1 SD, on the baseline, full-band stimulus condition of Experiment 1 (Fig. 2B) along with performance on the two split-harmonic conditions. When the split-harmonic filters were introduced with no delay offset of FM2 relative to FM1 (both at 0 μs), performance declined from a mean of 7.2% errors to a mean of 9.3% errors, which was a slight but statistically significant increase in errors ($P<0.05$). When FM2 is additionally delayed by 300 μs relative to FM1, performance declined further to 17.0%, which is significantly different than for the split-harmonic filters alone ($P<0.001$). Thus, while introduction of the split harmonic filters to segregate FM1 and FM2 produces a small decrease in delay acuity at 800 μs, it is not enough to obscure the much larger effects of disrupting the timing of the harmonics.

## EXPERIMENT 4: AMPLITUDE-LATENCY TRADING AND COMPENSATORY TIME SHIFT

**Procedure**

The results from Experiment 3 (Fig. 4B) reveal that it is feasible to use the split-harmonic technique to assess, first, the loss in acuity caused by changing the amplitude of one harmonic relative to the other, and second, the potential restoration of acuity caused by adding a countervailing change in the delay of one harmonic relative to the other. Two stimulus conditions were run in order to demonstrate the amplitude-latency trading effect, in which decreasing the amplitude of FM2 in echoes retards their perceived delay by 16 μs /dB, and its removal by advancing the delay of FM2 by an equal amount. The

spectrograms in Fig. 5A illustrate conditions for Experiment 4 along with two conditions from Experiment 3 for comparison. The first spectrogram on the left of Fig. 5A shows the baseline, full-band echo from experiment 1 used as S+. The second spectrogram shows the split-harmonic echo with 0 μs delay difference between FM1 and FM2 used as S+. The third spectrogram shows a split-harmonic echo with FM2 attenuated by 3 dB relative to FM1 used as S+ (first of two new conditions for Experiment 4). Based on the amplitude-latency trading ratio of −16 μs /dB, an attenuation of 3 dB should retard neural responses evoked by FM2 by about 48 μs relative to responses evoked by FM1. The fourth spectrogram in Fig. 5A shows the split-harmonic echo with FM2 reduced by 3 dB *and* advanced in time by 48 μs used as S+, to compensate for amplitude-latency trading. The spectrogram on the right shows the baseline, full-band echo with both FM1 and FM2 used as S- for all of the illustrated conditions.

**Results**

Figure 5B shows the results for the two new conditions in Experiment 4, along with two previous results for comparison. As shown earlier, performance with split harmonic echoes with 0 μs harmonic shift (Fig. 4B) was only slightly, albeit significantly, worse than performance with baseline, full-band echoes. When bats were presented with split-harmonic S+ echoes in which FM2 was attenuated by 3 dB relative to FM1, performance declined drastically to 29% errors due just to the 3 dB change in amplitude ($P<0.001$). However, shortening the delay of FM2 by 48 μs, while it is attenuated by 3 dB, counteracted the amplitude-latency trading effect and improved performance to 10.3%. Critically, the improved performance restores acuity to what occurs for split-

harmonic filtering without any additional amplitude or time manipulation (ns=P>0.05). It thus appears that big brown bats use the timing of neural responses to represent the harmonic sweeps, at least to achieve acuity for perception of echo delay.

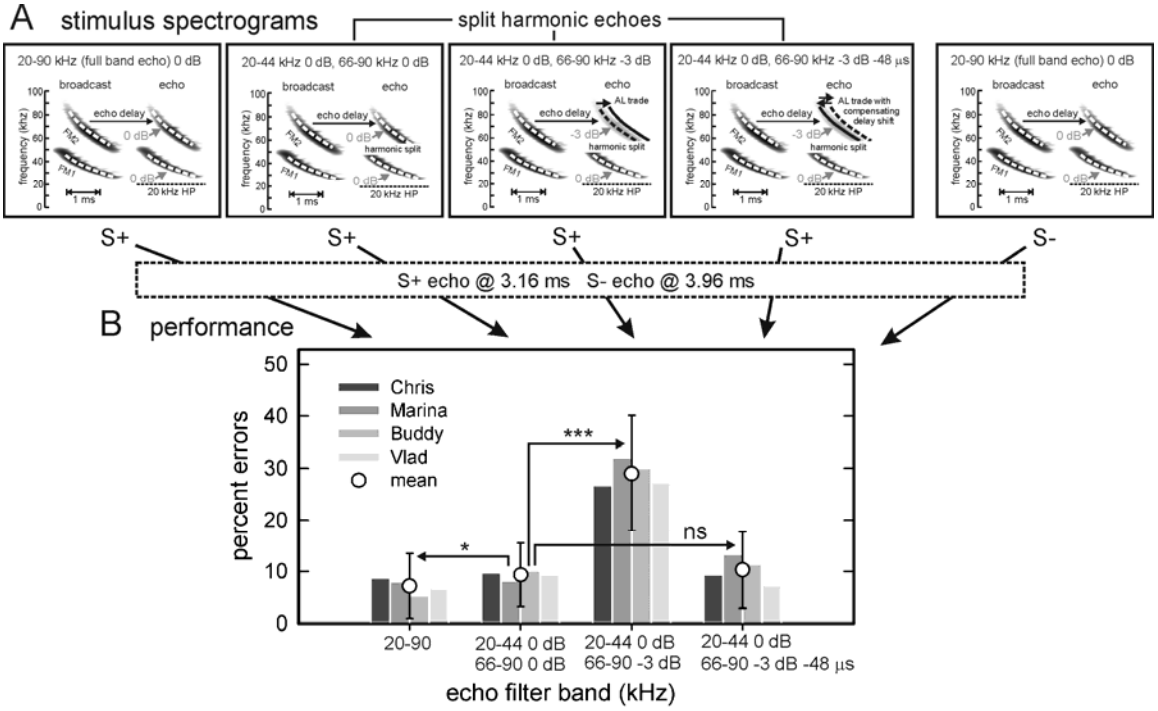


**Fig. 5** Experiment 4: Amplitude-latency trading and compensatory time shift: (*A*) Spectrograms for representative stimuli used to assess the loss in delay acuity if FM2 is decreased by 3 dB relative to FM1, and for testing the role of amplitude-latency trading by advancing FM2 in time by a compensatory 48 µs. (Amplitude-latency trading ratio for bats is -16 µs/dB.) The two spectrograms at left show full-band, normal echoes with *no* harmonic split for S+ and *with* harmonic split for S+ (from Fig. 5). Next two spectrograms show FM2 decreased in strength by 3 dB relative to FM1, or decreased by 3 dB and moved earlier in time by 48 µs. All S- are full-band echoes with no harmonic split (at right). (*B*) Bar graph showing performance on delay-discrimination tasks (percent errors) for 4 bats (150 trials/bat, with mean performance ±1 SD shown by circles). Introduction of harmonic-split filters with no other change causes small, significant decrease in acuity (more errors) compared to full-band echoes (* = P< 0.05). Decreasing FM2 by 3 dB causes further, large decline in performance (*** =P< 0.001), but advancing FM2 in time by 48 µs compensates for amplitude-latency trading and restores performance (ns = P> 0.05). Results show no evidence for perceptual effect from 3 dB loss of FM2 except for the associated latency shift.

# DISCUSSION

Taken together, the results of Experiments 1–4 confirm the expectation that big brown bats are sensitive to small perturbations of the FM harmonics in echoes (Hiryu et al., 2010). What is interesting here is that their sensitivity is manifested as a *loss in acuity* for perception of echo delay. Experiments 1–4 were not designed to determine whether bats can discriminate between echoes containing FM1 or FM2 in various combinations, but that these various combinations have effects on a fundamental perceptual dimension of echolocation—the delay of echoes.

Previous echo delay-discrimination experiments have used different types of echoes as stimuli, including electronically returned faithful replicas of each broadcast (as in the experiments described here) or "model" echoes, essentially a fixed, synthetic echo waveform triggered by the bat's sounds and presented at a controlled delay. Individual bat sounds will change from one broadcast to the next, but the model echo stays the same. Triggering of the model echo sound by each bat sound introduces significant timing jitter of 50 μs or more from one broadcast to the next, which limits the utility of model echo experiments to observing deterioration in performance beyond the 50 μs limit inherent in all two-choice delay-discrimination procedures. Given this limited accuracy of the two-choice method, bats have no more difficulty in discriminating the delay of model echoes (Masters and Jacobs, 1989) than the delay of real echoes from physical targets (Simmons 1971, 1973; Surlykke and Miller, 1985; Roverud and Grinnell, 1985) or from true electronic echoes that are delayed and attenuated versions of each of the bat's vocalizations (Denzinger and Schnitzler, 1994; Simmons, 1979, 1989). When certain parameters of model echoes are changed enough to exceed the 50 μs limit of the two-

choice method, bats do exhibit greater difficulty in discriminating their delays. For example, big brown bats can successfully *detect* model echoes presented either forward or reversed in time, but their delay discrimination threshold is normal (50–100 μs) only for echoes presented in their correct, forward direction; thresholds worsen substantially for time-reversed model echoes (Masters and Jacobs, 1989). Other studies have reported decrements in delay acuity only when the time-frequency structure of model echoes had been altered enough to matter (Surlykke, 1992; Masters and Raver, 2000). For example, when model echo duration, amplitude of FM2 relative to FM1, frequency sweep range, and curvature of frequency sweeps are systematically altered, the bats' performance was only affected by changing sweep shape (Masters and Raver, 2000). These experiments establish the plausibility of results that involve manipulations to deliberately make performance worse than the expected 50 μs limit of the two-choice method.

In Experiments 1–4 described above, the harmonics (FM1, FM2) in echoes that big brown bats received in two-choice delay-discrimination tests were electronically manipulated to assess their significance for perception of echo delay. A relatively large delay difference of 800 μs (3160 μs for S+ versus 3960 μs for S-) was used to ensure that any effects obtained by manipulating harmonics would not be a consequence simply of reducing echo bandwidth or changing echo center frequency. (In experiments using identical apparatus and stimulus levels, effects related to bandwidth and center frequency do occur, but on a much finer time scale of delay hyperacuity in fractions of a microsecond; Simmons et al., 2004). The 800 μs difference was used here to ensure that any changes in performance actually obtained would constitute a disruptive effect strong enough to spread over a span of time nearly a millisecond wide. It thus would represent a

significant loss of acuity to the bat. From Experiments 1–4, several manipulations, including truncation or removal of FM1and separation of FM2 from FM1 in time, yielded large decreases in the bats' performance (more errors) on the nominally easy 800 μs task, indicating that the loss in delay acuity associated with these manipulations was severe. Normal acuity in two-choice discrimination results is roughly 50 μs (Simmons, 1973; see Moss and Schnitzler, 1995; Simmons et al., 1995), but here the bat's acuity must have deteriorated to roughly 800–1000 μs, judging from the performance decrements obtained in the results described above. In spatial terms, this is equivalent to degrading the bat's normal, biologically quite useful distance acuity of about 1cm to a nearly-useless distance acuity of 16–20 cm. Several specific conclusions are warranted from these findings.

**Asymmetry in the Role of Harmonics**

The most obvious conclusion is that the two harmonics are asymmetric in their contributions to delay acuity. The results of Experiment 1 (Fig. 2B) show that big brown bats are sensitive in a graded manner to partial removal of FM1, and that performance falls to chance when FM1 is removed entirely. In contrast, complete removal of FM2 had only a slight effect on performance. Clearly, bats require the presence of FM1 to accurately perceive the delay of echoes, but the presence of FM2 is not required. Highpass filtering that removed small portions of the low-frequency end of FM1 disproportionately affected delay acuity (Fig. 2). Performance worsened when larger segments of low-end frequencies were removed. These results emphasize the importance of the low frequencies at the terminal end of FM1, which previous experiments have shown to be especially important for target detection (Bates et al., 2008). Results from

Experiments 1 and 2 are consistent with a previous finding that big brown bats can detect echo delay jitter as small as 4 μs equally well with FM1 and FM2 present together as with FM1 alone, but that the bats fail to perform the jitter task at all for echoes with FM2 alone (Moss and Schnitzler, 1989). As indicated by that earlier result and now also by the results of Experiment 2, big brown bats seem not to treat sounds containing only FM2 as being echoes at all. In Experiment 2, they prefer FM1 and FM2 together over FM2 alone, even when sounds containing just FM2 arrives full 800 μs earlier—a delay difference so large that the bats do not appear to judge FM2 by itself as even *having* a well-defined delay.

The auditory mechanisms underlying the behaviorally observed asymmetry in harmonic function must be far more complicated than just having responses to FM1 required for the determination of delay, with responses to FM2 adding only a little more information if FM2 is present. From Experiments 3 and 4, retarding the timing of FM2 relative to FM1 causes a significantly larger loss in acuity (17% errors for 300 μs offset introduced directly as in Fig. 4; 29% errors for 48 μs offset induced indirectly by amplitude-latency trading as in Fig. 5) than does total removal of FM2 (12.3% errors as in Fig. 2). The seemingly paradoxical result that misaligning responses to FM2 in time relative to responses to FM1 has a larger impact on performance than the complete absence of any responses at all to FM2 leads to a further conclusion. The bat's greater sensitivity to misalignment suggests that neuronal inhibition must be evoked by responses to frequencies in FM1, but must be applied to responses evoked by the higher frequencies in FM2. The proposed inhibition must be initiated by FM1 and must then persist over a short span of time following FM1. This inhibition normally would trail

behind simultaneously occurring excitatory responses to both FM2 and FM1, so that excitatory responses to FM2 would be finished before the inhibition caused by FM1 has started. However, retarding responses evoked by FM2 (either directly by shifting FM2 to a later time or indirectly by amplitude-latency trading when FM2 is attenuated) causes these responses to intrude into the inhibitory time window excited by FM1, which then leads to disruption of delay acuity.

The observed asymmetry of harmonic function seems to favor contributions from FM1 over contributions from FM2. To be clear, FM2 is not without function, it is just that its role is manifested here as causing a precipitous deterioration in delay acuity when retarded relative to FM1. The two-choice echo delay acuity observed in normal conditions appears to be due almost entirely to FM1 because graded removal of FM1 has corresponding effects on performance, while, when FM2 is totally absent, its contribution seems slight. The direction of the asymmetry revealed by Experiments 1–4 conforms to acoustic and auditory asymmetries normally experienced by echolocating bats, which leads to consideration of the purpose that is served by the asymmetry. (In a different type of experiment that probes the big brown bat's perception of delay on much finer time scale, graded removal of frequencies from either FM2 or from FM1 leads to a graded decrease in delay hyperacuity; Simmons et al., 2004. In those experiments, no abrupt deterioration occurs when FM1 is truncated. Both FM1 and FM2 are capable by themselves of supporting delay hyperacuity, which nevertheless is better when both harmonics are present).

**Harmonic Misalignment as a Mechanism for Rejection of Clutter**

Retarding of auditory responses to FM2 so they follow responses to FM1 causes the bats' delay acuity to decline so severely as to constitute a collapse of the bat's image along the delay axis. So extreme a consequence of merely shifting the auditory representation of FM2 by a small fraction of a millisecond, compared to the slight effect of complete removal of FM2, signifies that some important characteristic of echoes is bound up in the temporal relation between FM2 and FM1. Moreover, this characteristic must be important enough that the bat benefits from the resulting substantial loss of delay acuity for those sources. How might the bat gain advantage from deliberately creating a *loss* of acuity? Although true temporal lagging of FM2 after FM1 does not occur acoustically during sound propagation or reflection in air because sound velocities are the same for frequencies in both harmonics, *attenuation* of FM2 relative to FM1 due to lowpass filtering during propagation or from broadcast beaming *does* occur. In contrast, attenuation of FM1 relative to FM2 through highpass filtering is unlikely to occur because echoes from insect-sized targets have, on average, flat spectra over the frequencies used by big brown bats (Moss and Zageski, 1994; Simmons and Chen, 1989). When FM2 is weakened relative to FM1, amplitude-latency trading at a ratio of approximately −16 μs /dB (Bodenhamer and Pollak, 1981; Burkard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990) shifts responses evoked by FM2 into a time window that follows responses to FM1. The results of Experiment 4 (Fig. 5) establish unambiguously that a small (3 dB) reduction in the amplitude of FM2 causes retardation of responses through amplitude-latency trading, a retardation that can be offset by advancing the timing of FM2 itself. For these reasons, amplitude-latency trading is the only practical origin of temporal misalignment between harmonics experienced by bats. It

is the only realistic path for causing the bat's delay acuity to decrease by mechanisms identified from the results of Experiments 1–4.

What are the common features of objects whose echoes undergo lowpass filtering? The bat emits a broadcast containing FM1 and FM2 at approximately equal strength. The sounds that impinge on targets located directly in front of the bat and not too far away contain both of these harmonics at equal strength, too. But bats' broadcasts are emitted with a directivity that is dependent on frequency (Hartley and Suthers, 1989). The broadcast beam is very wide for frequencies of 20–30 kHz, present in the terminal end of FM1, but the beam grows narrower at higher frequencies, so that the frequencies of 55–90 kHz in FM2 are localized in a narrower zone in the center of the beam than the frequencies of 22–55 in FM1. As off-axis direction increases, the higher frequencies (e.g., FM2) are attenuated. Atmospheric attenuation also is stronger at higher ultrasonic frequencies. As target distance increases, FM2 gets progressively weakened relative to FM1 (e.g., Lawrence and Simmons, 1982). Thus, echolocation sounds impinging on targets located off to the sides or far away always undergo lowpass filtering, and echoes thus always return from objects in these locations with attenuation of FM2 relative to FM1. Due to amplitude-latency trading, the auditory representation of these echoes will include temporal misalignment of responses for FM2 relative to FM1, and correspondingly degraded acuity for perception of delay. In contrast, targets located immediately to the bat's front will be ensonified with both harmonics, and echoes will return with FM2 and FM1 at about the same strengths because the total distance of propagation is short. The practical significance of this distinction is that clutter—objects off to the sides or in the background—virtually always return lowpass echoes that, due to

relative attenuation of FM2, will be perceived with poor delay acuity. Echoes from any objects located in the frontal zone of immediate concern to the bat will be perceived as having well-defined delays. If this distinction is correct, then bats may have evolved low delay acuity as a means of preventing interference from clutter. It thus appears that FM2 may serve a specific perceptual purpose in the echolocation by big brown bats, and not a purpose encompassed just by the added bandwidth it represents.

## ACKNOWLEDGEMENTS

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CHAPTER 5

# Perception of echo delay is disrupted by small temporal misalignment of echo harmonics in bat sonar

Bates, M. E. and Simmons, J. A. (2011). Perception of echo delay is disrupted by small temporal misalignment of echo harmonics in bat sonar. *J. Exp. Biol*. **214**, 394-401.

## ABSTRACT

Echolocating big brown bats emit ultrasonic frequency-modulated (FM) biosonar sounds containing two prominent downward sweeping harmonics (FM1 and FM2) and perceive target distance from echo delay. In naturally occurring echoes, FM1 and FM2 are delayed by the same amount. Even though echoes from targets located off-axis or far away are lowpass filtered, which weakens FM2 relative to FM1, their delays remain the same. We show here that misalignment of FM2 with FM1 by only 2.6 μs is sufficient to significantly disrupt acuity, which then persists for larger misalignments up to 300 μs. However, when FM2 is eliminated entirely rather than just misaligned, acuity is effectively restored. For naturally occurring, lowpass filtered echoes, neuronal responses to weakened FM2 are retarded relative to FM1 because of amplitude-latency trading, which misaligns the harmonics in the bat's internal auditory representations. Electronically delaying FM2 relative to FM1 mimics the retarded neuronal responses for FM2 relative to FM1 caused by amplitude-latency trading. Echoes with either electronically or physiologically misaligned harmonics are not perceived as having a clearly defined delay. This virtual collapse of delay acuity may suppress interference

from off-axis or distant clutter through degradation of delay images for clutter in contrast to sharp images for nearer, frontal targets.

## INTRODUCTION

The biosonar sounds of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae), are frequency-modulated (FM) and contain several harmonics, the most prominent being the first (FM1; sweeping down from ~55 to 22 kHz) and the second (FM2; sweeping down from ~105 to 45 kHz) (Saillant et al., 2007; Surlykke and Moss, 2000). The bat's signals contain these harmonics when target classification or echo discrimination takes place and during interception, when accurate localization is necessary for tracking (Ghose and Moss, 2003; Ghose and Moss, 2006; Simmons et al., 1995). Furthermore, recordings of sonar signals actually impinging on a suspended target confirm that the sounds contain these harmonics throughout aerial interception maneuvers (Saillant et al., 2007). The presence of harmonics widens the effective bandwidth of the transmitted signals (Simmons and Stein, 1980), which increases the sharpness of biosonar images (Simmons et al., 1998; Simmons et al., 2004) and enhances the bat's ability to perceive objects close to background clutter (Siemers and Schnitzler, 2004).

There is new evidence that the harmonic structure of biosonar sounds may play a more specific role in echo processing than that which is evident from bandwidth considerations alone. Across the 22 to 105 kHz band encompassing both FM1 and FM2, sound velocity is constant, so frequencies in both harmonics arrive from a target at the same delay. That is, harmonics in naturally occurring echoes are coherent, with an exact

2:1 ratio of frequencies (FM1:FM2) from moment to moment throughout the FM sweep. When trained to discriminate electronically generated 'normal' echoes in two-choice tests, big brown bats exhibit a delay acuity of approximately 5̣0̣ μs (Moss and Schnitzler, 1995; Simmons et al., 1995). However, in new two-choice experiments with split-harmonic echoes, the bat's echo delay acuity is hugely disrupted – it deteriorates to approximately 80̣0̣ μs – if the principal harmonics, FM1 and FM2, are deliberately misaligned in time by 30̣0̣ μs or altered in relative strength so that neuronal responses to the harmonics are misaligned as a result of amplitude-latency trading (Bates and Simmons, 2010; Stamper et al., 2009).

In the present paper we measure the size of the smallest temporal misalignment of FM2 relative to FM1 that causes the big brown bat's delay acuity to undergo the severe deterioration that is already known to occur for a relatively large 30̣0̣ μs harmonic misalignment (Stamper et al., 2009). Using electronically generated split-harmonic stimuli, the time separation between FM2 and FM1 was decreased from 300 μs to 0̣ μs to determine the threshold for disruption of echo delay perception. There are two possibilities for how the disruption of acuity will be manifested. First, if the disruptive effect builds up gradually as the time interval between harmonics is increased, and even then only occurs for large harmonic misalignments (e.g. 30̣0̣ μs), it may not be particularly significant under ordinary conditions of echolocation because acoustic propagation in air cannot dissociate harmonics even by much smaller amounts. (The velocity of sound across the bat's echolocation frequencies is constant.) Second, if the disruption of delay acuity occurs abruptly for temporal misalignments much smaller than 30̣0̣ μs, it may prove to be an important aspect of echo processing, even under natural

conditions. Although harmonic misalignment cannot occur acoustically, it could occur internal to the bat's auditory system. Physiological mechanisms that retard neuronal response latencies (amplitude-latency trading) will create a wide range of different sized temporal misalignments of responses evoked by FM2 relative to FM1 because FM2 is normally attenuated more than FM1 in many echoes.

Many bats regularly fly and forage amidst clutter, such as dense vegetation, in conditions where the ability to perceive the space immediately to the front is crucial not only to locate targets but also to be sure no obstacles occupy this space (Jensen et al., 2001; Moss et al., 2006; Schnitzler et al., 2003; Siemers and Schnitzler, 2000; Siemers and Schnitzler, 2004; Simmons, 2005; Simmons et al., 2001). Echoes from surrounding clutter typically arrive at the same time as echoes from potential targets or obstacles located in the frontal zone and have to be classified as clutter. In an experiment investigating what is perhaps the worst case of dense, range-extended clutter, big brown bats were flown in an obstacle array made of large numbers of plastic chains hanging in rows from the ceiling of a flight room (Hiryu et al., 2010). The chain array was dense enough to require the bat to emit sounds at short intervals to update its images, and deep enough that echoes of each broadcast continued to arrive after the next broadcast was sent out. When all of the echoes from the first broadcast had not yet returned but the second sound was emitted anyway, the bat encountered potential pulse-echo ambiguity, defined as difficulty in determining which echoes belong to which emissions. Big brown bats solve this problem – that is, disambiguate the echoes to associate them with the corresponding emissions – by making slight changes of several kilohertz in the ending FM1 frequency of successive broadcasts. This subtle difference between successive

broadcasts (only a few percent of the total 80 kHz frequency band of the sounds) causes proportionately much larger changes in the time-frequency spectrograms of their echoes, which the bats evidently used to group the echoes with the correct broadcasts. From this result, the hypothesis for the experiments reported here is that big brown bats will be sensitive to disparities introduced into the time-frequency pattern of echo spectrograms. The simplest way to test whether bats are sensitive to small disparities in the spectrograms of echoes is to deliberately misalign the harmonics (separating FM1 and FM2 by controlled time intervals) and then to measure the size of the smallest misalignment that the bat can just perceive. The particular behavioral effect is, however, not whether bats can detect different degrees of misalignment, but the effect of misalignment on the acuity of echo delay-discrimination.

## MATERIALS AND METHODS

### Subjects

Four adult big brown bats (*Eptesicus fuscus,* Palisot de Beauvois 1796; three males and one female) were wild caught in Rhode Island with a permit from the state's Department of Environmental Management. They were housed in individual cages at 22–23°C and 50–60% relative humidity with free access to vitamin-enriched water. The day–night cycle of the room was reversed to 12h:12h dark:light so experiments could be conducted during the daytime. Bats' weights were maintained between 14.5 and 15.5g by adjusting the number of mealworms (*Tenebrio* larvae) that they were fed daily. Experiments complied with NIH Principles of Laboratory Animal

Care (NIH publication no. 86-23, revised 1985). Experimental procedures were approved by the Brown University Animal Care and Use Committee.

**Procedure**

The split-harmonic echo-delay discrimination experiment was run in a 4.0 m × 3.0 m × 2.5 m room with panels of sound-absorbent foam (SONEX®, Pinta Acoustic, Minneapolis, MN, USA) lining the floor and walls. Light levels were kept dim (~10 lux) during experimental trials to avoid disturbing the bats. The equipment for producing the electronic echo stimuli was located in an adjacent room. All trials were run double-blind; there were no visual cues available to the bats or to the trainers. The bats' task was to discriminate between electronically generated echoes that differed in arrival time while their harmonic structure was manipulated.

Each bat was trained to sit on an elevated Y-platform (Fig.1) and broadcast its echolocation sounds toward two ultrasonic microphones (Brüel and Kjær model 4138 1/8-inch condenser microphones, Nærum, Denmark), one located on each end of the platform. The signals picked up at these microphones were used to generate the echoes that served as stimuli for the experiments. Microphones were mounted 20 cm away and separated by 40 °. Echolocation sounds emitted by the bat were picked up by the microphones, filtered and delayed electronically and then delivered back to the bat from small electrostatic loudspeakers 15 mm in diameter (RCA, model 112343, Hauppauge, NY, USA). These speakers were mounted next to the microphones at the ends of both arms of the platform, 20 cm away from the bat and 50 ° apart. The stimuli delivered to the bat were delayed by 1160 μs through the combined acoustic paths from bat to

microphone and from the loudspeaker to the bat, plus electronic delays that varied from 2000 to 2800 μs according to conditions.

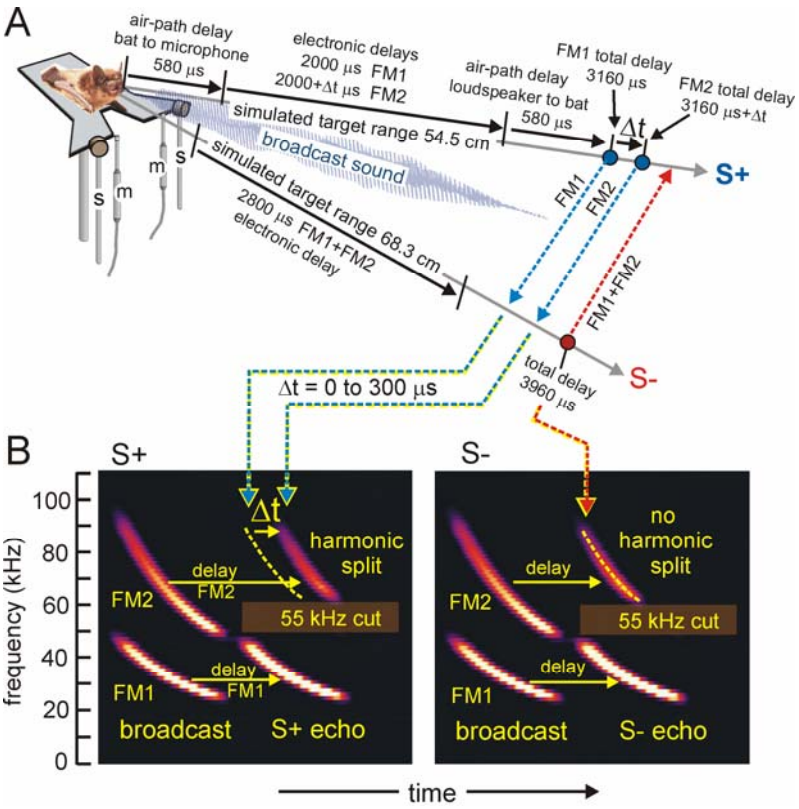


**Fig. 1.** Experimental method for assessing bat's perception of delay for split-harmonic echoes. (*A*) Diagram of two-choice procedure, with bat on Y-shaped platform. Electronic target simulator picks up bat signals at microphones (*m*), filters them to segregate the two harmonics, and returns them as delayed echoes from loudspeakers (*s*). (*B*) Representative spectrograms show bat's broadcast containing harmonics FM1 and FM2 with echo for S+ (left), and broadcast with echo for S- (right). Harmonics are split—FM1 by lowpass filters at 44 kHz and FM2 by highpass filters at 66 kHz, leaving a narrow band around 55 kHz cut out of both FM1 and FM2. For S+, the simulator delivers FM1 and FM2 at different delays, with small steps of added delay (Δt) from 0 to 300 μs for FM2 (dashed yellow curves on S+ echo). For S-, the simulator delivers FM1 and FM2 together at the same delay even though the harmonic split is present. The relatively large 800-μs delay difference between S+ and S- is the stimulus feature to be discriminated; the experiment measures the effects of different-sized harmonic misalignments (Δt) on performance.

The bat was rewarded with a piece of mealworm for walking down the arm of the Y-platform corresponding to the loudspeaker that delivered the positive stimulus (S+; see below), which was presented on either the left or right side in a pseudorandomized sequence (Gellerman, 1933). If the bat made an incorrect response, i.e. to the negative stimulus (S–; see below), a broadband sound was made to signal to the bat that it made an error, and a 5 s pause in the experiment ensued. All trials were run using a double-blind procedure. Two experimenters were present while each bat was run: a trainer who handled the bat and was blind to the position of the correct choice, and a recorder who controlled which loudspeakers generated the stimuli and recorded the bat's response. The recorder observed the bat using a black and white CCD video camera (Supercircuits, Inc., Type 166 15-CB22-1, Austin, TX, USA) mounted on the ceiling above the Y-platform. Illumination for the camera was provided by two infrared LED panels (Supercircuits, Inc.) located on either side of the video camera. The recorder was able to monitor the bat's performance on a Sony digital 8-mm video Walkman® recorder (New York, NY, USA) located behind the trainer and the Y-platform.

Sets of 50 experimental trials were conducted each day, with a total of three days for every condition. Each bat thus completed 150 trials over three days for each stimulus condition, for which the percentage of correct responses was recorded. All data were analyzed using binomial probability tests, which are the appropriate way to process data from independent trial, two-alternative forced-choice experiments such as those used here (SPSS v. 16.0, Chicago, IL, USA).

**Electronic stimuli**

The two-channel electronic target simulator system has been described fully elsewhere (Bates and Simmons, 2010) [see fig.4 in Stamper et al., 2009)]; no changes were made in the acoustic or electronic components for these new experiments. The stimuli were electronically generated echoes of the bat's own biosonar broadcasts. They differed in delay, which was regulated by digital electronic delay lines, and in harmonic structure, which was regulated by electronic filters in series with the delay lines (see below). The basic procedure was to present bats with both S+ and S– that were designed to incorporate split-harmonic echoes into an ordinary two-choice echo delay-discrimination protocol. For all conditions, S– consisted of a single-glint echo at a fixed delay. (A single-glint echo is a single reflected replica of the broadcast at a specific delay. A two-glint echo contains two reflected replicas at slightly different delays.) S+ for the experimental condition consisted of a split-harmonic single-glint echo with varying amounts of time separation between the harmonics. S+ for the control condition was a two-glint echo with the first glint at the same delay as FM1 in the split-harmonic condition and the second glint at the same delay as FM2. Thus, experimental data were collected using time separations between FM1 and FM2 that could be compared with data collected using the same time separations between two glints. Although two-glint echoes commonly occur (Simmons and Chen, 1989), split-harmonic echoes would not occur in nature because the velocity of sound is the same at all bat frequencies. Nevertheless, electronic manipulation of harmonic delays is used here as a tool to examine the bats' sensitivity to disruption of echo spectrograms on the time-frequency plane. Bats perceive the delay for each glint in a two-glint echo for separations larger than 2 μs (Simmons et al., 1998), but the errors bats make in this task are confined to a

narrow span of delays close to the glint delays themselves. In split-harmonic experiments (Bates and Simmons, 2010; Stamper et al., 2009), bats make errors over a wide span of delays extending hundreds of microseconds away from each harmonic delay. For this reason, in the present study two-glint stimuli were used as control stimuli so that all the effects of electronic delays, filtering and simulation of echoes from loudspeakers could be combined to create data with which to assess the added effects of the harmonic misalignment.

The stimuli are best described in terms of changes imposed on the signals between their being picked up by the microphones and their being broadcast back to the bat from the loudspeakers. After being picked up by the left and right microphones (Fig.1), the bats' signals passing through the left and right simulator channels were highpass (HP) filtered at 20̣ kHz (Rockland model 442 variable electronic filters; 36̣ dḄ octave–1; San Diego, CA, USA) and lowpass (LP) filtered at 90̣ kHz (Stewart model vbf-8 variable electronic filter; 48̣ dḄ octave–1; Beckenham, Kent, UK) to set the overall boundaries of the echo band delivered to the bat and to remove low frequency background noise. In each simulator channel, these filtered signals were then delayed by specially built digital delay lines (1.3 μs delay steps, 10 bit digitizing accuracy from analog input to digital delay and back to analog output). There were two parallel electronic delay lines in each simulator channel (left and right). The output of one was HP filtered and the output of the other was LP filtered (Rockland model 753A variable electronic filters; 112̣ dḄ octave–1). The delayed HP and LP filtered signals in each simulator channel were then summed by an analog mixer, again LP filtered at 90̣ kHz

(Rockland model 442 variable electronic filters; 36 dB octave–1), and finally delivered to the left and right loudspeakers (RCA type 112343), which returned them to the bat as acoustic echoes.

The stimuli in the experimental and control conditions differed in the delay settings of the two delay lines in each simulator channel and in the HP and LP filter frequencies. In the experimental condition (split-harmonic S+; Fig.1B), the LP filter in each simulator channel was set to 44 kHz to select only FM1 from the signals whereas the HP filter was set to 66 kHz to select only FM2. A narrow frequency band around 55 kHz was removed (cut) entirely from both S+ and S– to eliminate frequencies at which FM1 and FM2 might overlap. For experimental S+, the overall delay of the filter-selected FM1 was set to 3160 μs (2000 μs electronic delay + 1160 μs sound path delay, corresponding to a target distance of 54.5cm) whereas the delay of the filter-selected FM2 was increased above 3160 μs by varying amounts of electronic delay between 2300 and 2000 μs. The split-harmonic delay difference between FM1 and FM2 ($\Delta t$) thus varied over steps of 300, 100, 25, 5.2, 2.6, 1.3 or 0.0 μs. For experimental S–, the overall delays of the filter-selected FM1 and FM2 were set to the same value of 3960 μs (2800 μs electronic delay + 1160 μs sound path delay, corresponding to a target distance of 68.3cm). Under ordinary conditions, this 800 μs delay difference is easily discriminated by big brown bats (Moss and Schnitzler, 1995; Simmons et al., 1995). The crucial feature of the experimental condition is that both S+ and S– undergo the same electronic filtering; only the electronic delays of FM1 and FM2 differed, by varying amounts of $\Delta t$ for S+ and by 0 μs for S–.

In the control condition (two-glint S+), the LP filter in each simulator channel was set to 90̣ kHz whereas the HP filter was set to 15̣ kHz, to allow both FM1 and FM2 to pass unobstructed into the echoes returned to the bat. For control S+, the overall delay of the first glint was set to 316̣0 μs (200̣0 μs electronic delay + 116̣0 μs sound path delay) whereas the delay of the second glint was increased to >316̣0 μs by varying amounts of electronic delay between 2300 and 2000 μs. The two-glint delay difference (Δ*t*) thus varied over steps of 300, 100, 25, 5.2, 2.6, 1.3 or 0.̣0 μs. For control S–, the overall delay of the two glints was set to the same value of 396̣0 μs (280̣0 μs electronic delay + 116̣0 μs sound path delay). The crucial feature of the control condition is that both S+ and S– again undergo the same electronic filtering; only the electronic delays of the two glints differed, by varying amounts of Δ*t* for S+ and by 0̣ μs for S–.

The time differences between FM2 and FM1 (Δ*t*) for the harmonic split were the same as those used for the two-glint separation, so that comparison of the bats' performance in split-harmonic and two-glint conditions would reveal the effects of the harmonic delay separation. Besides the varying time differences for FM2 relative to FM1, or between the first and second glints, the only difference between the experimental and control conditions was the presence of the harmonic split filter settings (i.e. the narrow filtered region around 55̣ kHz removed from the split-harmonic stimuli; see Fig.1B). The effects of this 55̣ kHz cut can be evaluated by comparing the performance of the bats under experimental and control conditions at Δ*t*=0̣ μs. If their performance is the same under both conditions, then the split-harmonic filters had no effect of their own beyond the delay differences between FM2 and FM1.

From bat sound at the microphone to its echo at the bat's location on the platform, the total gain of the target simulator system was approximately –35̣ dB for S+ and –40̣ dB for S–. The big brown bat's sensitivity for echo detection is not a fixed quantity; thresholds for echo detection are high immediately following the broadcast and decline by approximately 11̣ dB per doubling of delay thereafter for at least 6–10̣ ms (Kick and Simmons, 1984). The 5̣ dB attenuation of S– relative to S+ in the present study compensates for the 5̣ dB increased sensitivity of the bat for echoes at the longer delay of 396̣0 μs compared with the shorter delay of 316̣0 μs.

## RESULTS

Fig.2A plots the results of the present experimental and control conditions relative to data previously collected with respect to echo delay acuity in big brown bats. These curves all show mean percentage errors achieved by multiple bats. First, plotted in green, is a complete two-glint performance curve that shows percent errors made on an echo delay-discrimination task where S+ is a two-glint echo at a delay of 3.2̣ ms (300 μs glint separation) and S– is a single-glint echo at various delays from 3.1 to 3.9̣ ms (mean performance on 60 trials per bat, *N*=2 bats). [These data are from previous experiments (Simmons et al., 1990b); that they were not plotted in the original paper is solely due to limitations of space.] In that experiment, the delay of S– was moved along the horizontal S–/S+ delay difference axis (Fig.2A) to probe for locations (delays of S–) where bats would perceive S– and either glint of S+ as having the same delay. The discrete error peaks at 0 and 30̣0 μs (green curve) show that the bats separately perceive the delay of each reflection in the two-glint S+.

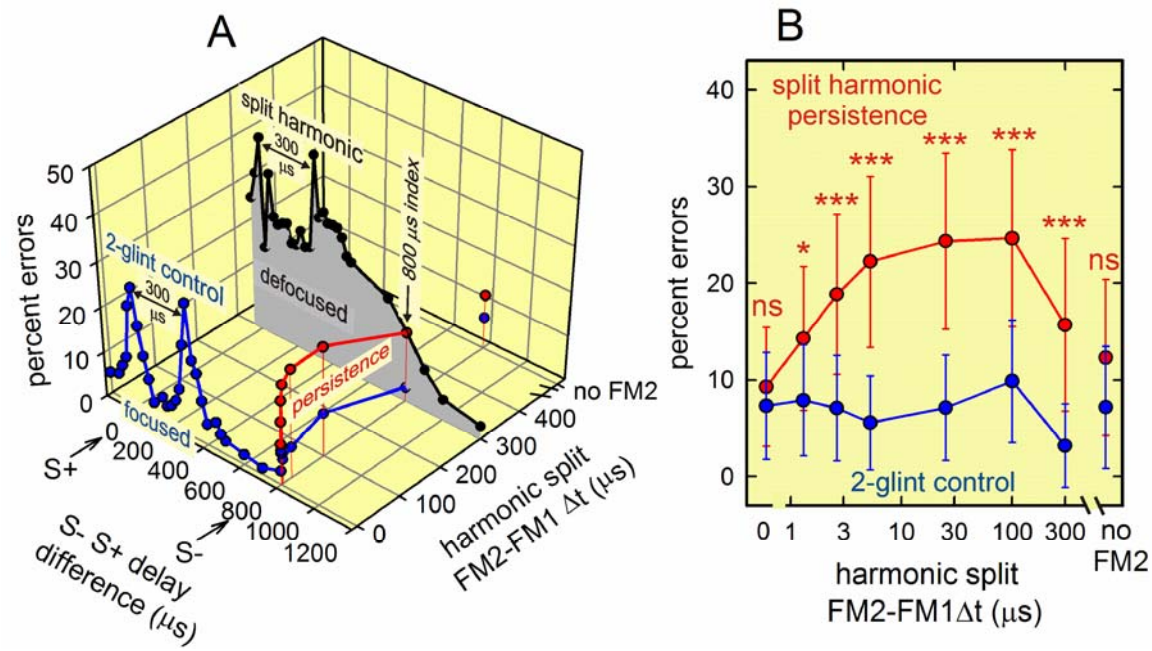


**Fig. 2.** Image defocusing caused by harmonic misalignment. (*A*) Explanation of defocusing: Blue two-glint control curve shows percent errors (mean of 4 bats) in delay discrimination tests with S+ as two-glint echo having 300-µs glint separation, and S- as 1-glint echo. Harmonic split (Δt) is 0 µs. Discrete error peaks at 0 and 300 µs (in blue) show that the bats separately perceive the delay of each reflection in the 2-glint echo. Black curve with gray shaded area shows percent errors (mean of 4 bats) for same task but with S+ as split harmonic echo having FM2 at 300-µs longer delay than FM1. Again, S- is 1-glint echo presented at different delays to probe for perceived delays in S+. Error peaks at 0 and 300 µs (in black) show that the bats separately perceive the delay of each harmonic (FM1 at 0 µs, FM2 at 300 µs), but these peaks appear as modulations on a broad pedestal of errors (gray area) stretching from 0 µs to over 800 µs. The pedestal of errors illustrates a blurred or *defocused* delay image, whereas the well-defined peaks in the 2-glint curve (blue) illustrate a *focused* image that resolves the two glints. In the present experiment, the bat's performance at a normally easily-discriminated delay difference of 800 µs between S+ and S- is used as the index of defocusing while the harmonic split (Δt) is changed in steps from 300 µs down to 0 µs (red). Significant defocusing of the bat's delay image begins for harmonic offsets as small as 2.6 µs and persists for offsets up to 300 µs. If FM2 is not just delayed but removed entirely, performance returns to normal for focused images. (*B*) Mean performance (± 1SD) of 4 bats on 800-µs delay discrimination task (index, in A) in harmonic split experiment (red) and in 2-glint control (blue). (ns= P> 0.007; * =P< 0.007 by binomial tests)

Results from split-harmonic electronic echoes using an S+ with a 300 μs offset of FM2 relative to FM1 are plotted in black (Fig.2A) [150 trials per bat, *N*=4 bats; re-plotted from Stamper et al., 2009]. For the S+ in that experiment, FM1 delay was 3160 μs and FM2 delay was 3460 μs. Again, S– is a one-glint echo presented at different delays to probe for perceived delays associated with each of the harmonics in S+. This black error curve has prominent error peaks at delays of S– that correspond to the delay of either FM1 or FM2 in S+ (at 0 and 300 μs), indicating that the bats perceived the delay of each harmonic separately. However, instead of error peaks sharply localized to the immediate region of the delay for FM1 or FM2, as occurs for the two-glint curve (green), there is a broad pedestal of errors (gray shaded area) surrounding these peaks and extending continuously from the arrival time of FM1 to longer delays past 800 μs. This pedestal of errors extends more than 800 μs past the arrival time of FM2, which itself is only 300 μs after FM1. The pedestal is referred to here as a blurred or defocused delay image because the bats' performance reveals a lack of sharply perceived delay for the 300 μs split-harmonic stimuli. By contrast, the well-defined peaks in the two-glint (green) curve illustrate a focused image that resolves the two glints as having separate delays.

The present experiment with a variable offset of FM2 relative to FM1 addresses the sensitivity of the perceptual mechanism underlying the defocusing effect illustrated by the gray area in Fig.2A: how small a misalignment of FM2 from FM1 is sufficient to initiate the pedestal of errors? A stimulus delay difference of 800 μs is used as an index of blurring because it is remote from any of the ordinary effects of glints separated by 300 μs (green curve).

In Fig.2 A and B, the red and blue curves (split-harmonic persistence and two-glint control) are the mean split-harmonic experimental (red) and two-glint control (blue) data (mean ± SD percent errors from 150 trials per bat, *N*=4 bats) from the present experiment. The mean results are plotted on Fig.2A on the linear scale of the time separation (Δ*t*) of FM2 from FM1 from 300 to 0 μs. An additional data point is shown for the condition of no FM2 (i.e. only FM1; Bates and Simmons, 2010). These same data are replotted in Fig.2B on a logarithmic scale for the separation (Δ*t*) of FM2 from FM1 to spread out the abrupt onset of the loss in performance, or defocusing effect, when FM2 is delayed by only a few microseconds relative to FM1. A Bonferroni correction resulted in a significant alpha level of 0.007. There was a significant decrement in performance (increase in percentage errors) for the experimental echoes compared with the control echoes beginning with the 2.6 μs harmonic separation. That is, a significant defocusing effect already is evident at the 2.6 μs harmonic separation, and it increases further as harmonic separation increases to 25 μs and greater. This defocusing effect then persists as Δ*t* increases to 300 μs. It was only at the 0 and 1.3 μs harmonic separation that the mean performance of the bats was statistically indistinguishable between conditions (Fig.2B). Comparison of experimental and control performance at the stimulus condition of Δ*t*=0 μs tests for the effects of the split-harmonic filters themselves (55 kHz cut; Fig.1B); the statistical similarity of performance indicates that the experimental and control results are not influenced by a bias associated with these filter settings.

When FM2 was removed entirely from S+ echoes, instead of being delayed relative to FM1, mean performance then returned to being statistically indistinguishable

between control and experimental conditions (Fig.2B). The delay acuity of the bats did not seem to be compromised when only FM1 was present in returning echoes.

To summarize, when the temporal relationship between FM1 and FM2 is gradually disrupted through shifting the arrival time of the two harmonics by $\Delta t$, the bats abruptly lose acuity for performing the nominally easy 80̣0 μs delay-discrimination task through a process that amounts to defocusing of the image (Fig.2A). Misalignments of FM2 with FM1 as small as 2.6 μs cause the occurrence of significant defocusing (Fig.2B).

## DISCUSSION

The results described above show that big brown bats perceive echoes with misaligned harmonics – even echoes misaligned by as little as 2.6̣ μs – as having a poorly defined delay. This effect is described here as defocusing of the bat's delay image. Use of the term defocusing is justified by the wide spread of poor performance, extending out to 80̣0 μs longer than the objective arrival time of the echoes. By contrast, two-glint echoes with glint separations ($\Delta t$) from 0 to 30̣0 μs (equal to the harmonic separations in Fig.2) are perceived with very few errors in the 80̣0 μs discrimination task because 800 μs is so remote from the delay of either the first or the second glint (green curve; Fig.2A). Most importantly, two-glint echoes with $\Delta t$ from 10 to 30̣0 μs are perceived as having two glints at their corresponding delays (Simmons et al., 1990a; Simmons et al., 1995; Simmons et al., 1998). The green two-glint control curve in Fig.2A illustrates how the two-glint image is in focus; i.e. the glints are registered separately at their correct delays,

with a narrow spread of errors (width of green error peaks) over approximately ±50 μs. The intervening space does not contain many errors and keeps the peaks separate.

Several studies involving a different approach than using electronically generated echoes have reported decrements in delay discrimination performance when the time-frequency structure of echoes is altered in ways other than temporal misalignment of harmonics (Masters and Jacobs, 1989; Masters and Raver, 2000; Surlykke, 1992). These experiments used an electronically generated model echo – a single transmitted waveform selected from a series of each bat's sounds and stored digitally to replace actual echoes of individual broadcasts. This model echo is triggered by the bat's sounds to arrive at a fixed delay for a delay-discrimination task (two-choice or go/no-go) (see Moss and Schnitzler, 1995). In various model echo tests, the parameters of echo duration, amplitude of FM2 relative to FM1, signal frequency and the curvature of FM sweeps (adjusted by changing the decay constant of an exponential curve used as the modulation function) were changed to assess the effect on delay-discrimination acuity. Besides the dramatic loss in acuity caused when echoes were reversed in time so that they swept upward in frequency instead of downward (Masters and Jacobs, 1989; Surlykke, 1992), only changes in the curvature of the FM sweeps yielded changes in performance that could be detected with the discrimination procedure (Masters and Raver, 2000). In the context of the present results, this is the only manipulation that led to differences in the spectrograms of echoes relative to broadcasts comparable to the differences produced in split-harmonic echoes (Fig.1B).

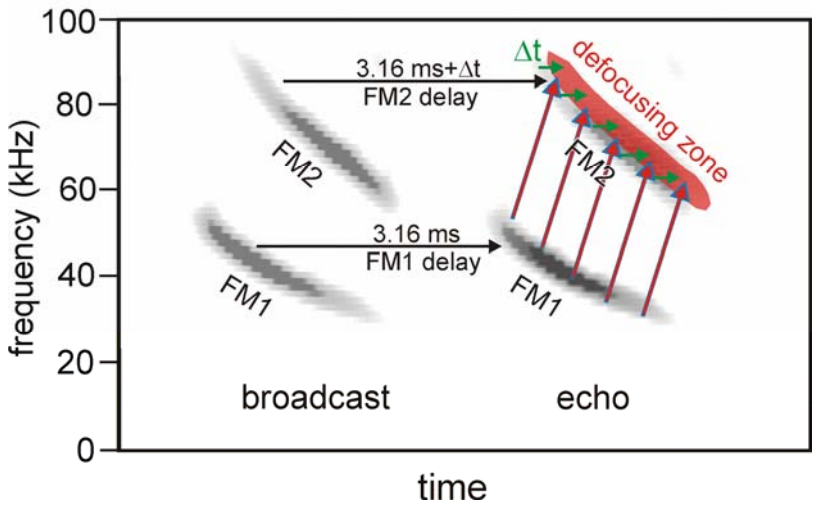


**Fig. 3.** Region of harmonic misalignment for image defocusing. Spectrograms of broadcast (left) and echo (right) showing harmonic sweeps, FM1 and FM2, and the bat's representation of echo delay from the time that elapses between broadcast and echo spectrograms (horizontal black arrows). When FM2 is offset in time (Δt) relative to FM1 (Fig. 2B), the bat's delay acuity deteriorates, but it recovers if FM2 is removed entirely. Neural responses evoked by FM2 that fall in a time window after the "correct" time aligned with FM1 interact with responses triggered by FM1 to blur the bat's delay image. Onset of defocusing is abrupt—it reaches significant strength for only 2.6 μs harmonic offset, and it persists for at least 300 μs.

The diagram in Fig.3 addresses implications of the defocusing effect caused by misaligning harmonics in echoes. A target represented by split-harmonic echoes would be perceived as being badly smeared along its echo delay axis, or as having a defocused distance and shape. By contrast, a target represented by echoes with correctly aligned harmonics, or echoes that contain FM1 alone, would be perceived as having a well-defined distance and shape. That is, its image would be in focus. The bat's sharp threshold sensitivity (2.6 μs), along with the persistence of defocusing for all values of harmonic separation from 2.6 to 300 μs, implies that some auditory mechanism detects harmonic misalignment of any size and then imposes strong defocusing on the resulting images. The virtual disappearance of defocusing when FM2 is removed completely, in contrast to the persistence of defocusing for various harmonic misalignments, argues for

a zone of time immediately following the nominal, or correct, time-of-occurrence of the FM2 sweep relative to the FM1 sweep, within which the anomalous presence of the FM2 sweep initiates the defocusing effect. That is, the correct alignment of FM2 with FM1 is not being detected; otherwise, removal of FM2 would cause defocusing to occur. Instead, the misalignment of FM2 over a range from 2.6 μs to at least 300 μs is being detected. The crucial zone of time for misalignment begins almost immediately (i.e. 2.6 μs) following the nominal position of FM2 and extends to at least 300 μs later without serious deterioration of the defocusing effect. This pattern of results suggests that, when responses to FM2 are allowed to shift to a later time than normal, they activate neuronal inhibition that initiates a cascade of responses designed to register the shape of targets from echo interference spectra (see Sanderson and Simmons, 2000; Sanderson and Simmons, 2002).

What is the significance under natural conditions of defocusing caused by temporal misalignment of echo harmonics if the atmosphere is a nondispersive medium for propagation of sound, especially over the short distances of up to 10 m that are relevant for echolocating bats? Biosonar in air is dominated acoustically not by differences in the velocity of sound across frequencies but by LP effects related to propagation and directional beaming. First, atmospheric absorption attenuates higher frequencies relative to lower frequencies in proportion to target distance. The degree of LP filtering present in the outward-propagating sound increases with distance, and then it doubles over the echo's return path (Lawrence and Simmons, 1982). For a target at a distance of 3 m, for example, frequencies of 50–100 kHz in FM2 would undergo excess attenuation from atmospheric absorption by 9 to 18 dB whereas frequencies of 25–50

kHz in FM1 would be attenuated by <4 to 9 dB. Consequently, FM2 would be attenuated by approximately 5 to 9 dB more than FM1. Second, the bat's broadcasts are projected in a beam towards the front, with sharper directionality at high frequencies than at low frequencies (Ghose and Moss, 2003; Hartley and Suthers, 1989). The greater the eccentricity, or off-axis position, of the target, the more the incident sound undergoes LP filtering before it impinges on the target. The degree of LP filtering in echoes thus increases with both distance and off-axis position. In relation to harmonic structure, echoes from targets located straight ahead and at short range reach the bat's ears with FM1 and FM2 largely at the same strength, but as target range or off-axis location increases, FM2 is disproportionately attenuated in echoes relative to FM1. The opposite effect – HP filtering to remove FM1 so that echoes contain primarily FM2 – does not occur under natural conditions because the dimensions of flying insects and virtually all other objects, such as leaves, branches or the ground, are larger in proportion to the incident wavelengths than the Raleigh region for scattering (Fenton et al., 1998; Houston et al., 2004; Moss and Zageski, 1994; Simmons and Chen, 1989). That is, natural targets create echoes in an acoustic regime where the full bandwidth of incident sounds returns at an amplitude related to the target's crosssectional area (the target's acoustic 'size').

Insect-sized targets affect echo spectra, but not as global LP filtering. The target's contribution instead consists of local notches in the spectrum due to reinforcement and cancellation caused by interference between overlapping reflections from multiple parts of the target, or glints (the target's acoustic 'shape') (Kober and Schnitzler, 1990; Moss and Zagaeski, 1994; Simmons and Chen, 1989). Behavioral, neurophysiological and computational studies have identified a process, called spectrogram correlation and

transformation (SCAT), that has been hypothesized to explain how big brown bats locate the frequencies of these interference notches and reconstruct the corresponding delay differences between different parts of the target (Matsuo et al., 2004; Neretti et al., 2003; Peremans and Hallam, 1998; Saillant et al., 1993; Sanderson and Simmons 2000; Sanderson and Simmons, 2002; Sanderson and Simmons, 2005; Simmons et al., 1995; Simmons et al., 1998). Using a combination of overall echo delay and the echo interference spectrum as cues, these bats recreate for each echo an image depicting the object as a small number of glints on a perceptual axis of distance.

In contrast to specific shape-related spectral signatures caused by interference between glints, the presence of LP filtering can reliably be exploited to distinguish all echoes reflected by off-axis or distant clutter from echoes reflected by targets of interest located to the immediate front of the bat. How is this distinction represented in the bat's auditory system? Neurons at successive stages (cochlear nucleus to auditory cortex) in the big brown bat's auditory system are tuned to different frequencies across the echolocation band of roughly 20–100 kHz (Dear et al., 1993a; Dear et al., 1993b; Ferragamo et al., 1998; Haplea et al., 1994; Jen et al., 1989; Ma and Suga, 2008; Pollak et al., 1977; Sanderson and Simmons, 2000; Sanderson and Simmons, 2002). Results of these experiments document a basic echo delay processing scheme that uses dispersed response latencies as delay lines in the inferior colliculus followed by coincidence-detecting neurons in the auditory cortex. Delay-tuned neurons created at the auditory cortex by this processing cascade respond at a wide range of latencies. The only extraneous influence on the accuracy of echo delay coding is amplitude-latency trading, a consequence of increased response latencies for echoes that are reduced in amplitude

(Bodenhamer and Pollak, 1981) [in big brown bats, the trading ratio is approximately –16 μs/dB (Burkhard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990a; Simmons et al., 1990b)]. The majority of delay-tuned neurons in the cortex are selective not only for a particular echo delay but also for the presence of an interference notch at their tuned frequencies. That is, although inferior colliculus neurons fail to respond if an interference notch is present at their tuned frequency (Sanderson and Simmons, 2000; Sanderson and Simmons, 2005), cortical neurons only respond if there is a notch at their tuned frequency, an inversion of the neuronal representation that depends on the evocation of inhibition by the 'missing' responses in the inferior colliculus (Sanderson and Simmons, 2002). Individual delay-line cells in the inferior colliculus produce an average of only one spike for each broadcast or echo (Sanderson and Simmons, 2000; Sanderson and Simmons, 2005), so if a particular frequency is reduced in strength in an echo due to interference, the single spikes evoked in neurons tuned to that frequency are retarded in latency by amplitude-latency trading (Burkhard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990b). When some frequencies in echoes are reduced in amplitude by interference, the spikes representing these frequencies thus shift to longer latencies up to several hundred microseconds following the latencies at which they would have responded if no reduction in amplitude had occurred. The resulting temporal misalignment of responses at specific frequencies in the inferior colliculus may initiate the inhibitory process that inverts the notch representation and displays the glints in the cortex (Sanderson and Simmons, 2002). The size of the time window containing neuronal responses that have retarded latencies due to interference notches thus coincides with the time window (Fig.3) into which the shift of FM2 causes defocusing of the delay image.

We speculate that the time-sensitive inhibitory process the big brown bat uses for registering individual interference notches is also activated by intrusion of responses evoked by electronically delayed FM2. However, although interference notches are distributed systematically across several discrete frequencies in echoes according to the time separation of the glint reflections (Moss and Schnitzler, 1995; Simmons et al., 1995), we hypothesize that the shift of the entire range of frequencies in FM2 into the inhibitory time window causes many different notch-selective neurons to be activated simultaneously, in effect 'turning on' a large number of perceived glints and defocusing the image. Delay separation of FM2 from FM1 does not occur acoustically in natural echoes. However, the decline in amplitude of FM2 relative to FM1 due to off-axis location or long target range leads to greater amplitude-latency trading of responses evoked by FM2. In effect, the auditory representation of FM2 is split from the auditory representation of FM1. For example, the LP effect described above for echoes arriving from a target at a range of 3̣ m results in attenuation of frequencies in FM2 by 5 to 9̣ dB relative to frequencies in FM1. The corresponding retardation of responses to FM2 due to amplitude-latency trading (at 16̣ μs /dB attenuation) is 80 to 14̣0 μs, which is solidly within the time window for defocusing of the bat's delay image (Fig.2B).

The bat's delay images for targets located straight ahead and at short range will have high delay accuracy and resolution because the only spectral effect is interference due to the glint structure of targets, not a global LP effect. By contrast, images of background objects, or clutter, located farther away or off to the side will be rendered with very low delay acuity due to defocusing caused by LP filtering, which retards the auditory representation of FM2 relative to FM1. The imaging process in biosonar thus

may be analogous to high-resolution foveal vision and low-resolution peripheral vision, except that objects are rendered in depth, not in direction.

## ACKNOWLEDGEMENTS

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CHAPTER 6

# Clutter rejection in bat sonar is achieved by image defocusing from harmonic beamforming

Bates, M. E., Simmons, J. A. and Zorikov, T. V. (2011). Clutter rejection in bat sonar is achieved by image defocusing from harmonic beamforming. *Science*. In press.

## ABSTRACT

When bats use biosonar to fly in cluttered surroundings, echoes that arrive from objects located to the sides mask perception of objects to the front, causing "blind spots." Due to broadcast beaming, echoes from the sides or farther away are lowpass-filtered, which attenuates the 2nd harmonic relative to the 1st harmonic. Neural responses to 1st and 2nd harmonics then become misaligned by amplitude-latency trading, which disrupts echo-delay acuity but also suppresses masking. Delay acuity and masking are restored when responses to echo harmonics are electronically realigned to offset amplitude-latency shifts. Bats use the harmonic structure of their sonar beam to delineate a frontal region of space for focused delay acuity surrounded by a region of defocused acuity combined with reduced clutter interference from lowpass echoes.

## BACKGROUND

For biosonar (Au and Simmons, 2007), big brown bats (*Eptesicus fuscus*) transmit brief frequency-modulated (FM) sounds covering 23 to 100 kHz with two prominent harmonics—FM1 and FM2 (Fig. 1A) (Saillant et al., 2007; Surlykke and Moss, 2000).

Although known for aerial pursuit of insects, they also fly through vegetation in transit from place to place, when capturing prey on vegetation, and when chasing each other (Simmons, 2005; Simmons et al., 2001). Vegetation consists of clusters of reflecting surfaces (leaves, branches) at varying distances that reflect numerous echoes at different delays (Muller and Kuc, 2000; Yovel et al., 2008). Surrounding objects constitute *clutter*. Echoes from clutter can cause masking of echoes from straight ahead, creating "blind spots" that compromise perception of targets (Fig. 1B). Because broadcasts are widely beamed, strong echoes are received from all directions. Nevertheless, big brown bats can fly through dense arrays of obstacles, even when space to maneuver is less than 1 m (Hiryu et al., 2010; Petrites et al., 2009). We report new experimental results that address how bats perceive objects to the front in spite of surrounding clutter.

Bat biosonar sounds are broadly beamed (Fig 1C). Horizontal beamwidth is frequency-dependent, ranging from ±70° at 25 kHz in FM1 to ±30° at 80 kHz in FM2 (Ghose and Moss, 2003; Hartley and Suthers, 1989). When the bat tracks a target (±1°-5° head-aim accuracy; Masters et al., 1985; Surlykke et al., 2009), that target is ensonified with the full broadcast band. However, the beam ensonifies surrounding clutter, too. Sounds that impinge on objects located off the beam's axis are weakened but also lowpass-filtered (Fig. 1C). Echoes from objects at longer ranges are reduced in overall amplitude by spreading losses but also lowpass-filtered by frequency-dependent atmospheric attenuation (Fig. 1D). (Acoustical Society of America, 1995). FM2 is weaker than FM1 in echoes from off axis or greater ranges compared to the original broadcast or to echoes from nearby targets located straight ahead. (The target's contribution comes from interference between multiple reflections returned by individual

parts, or *glints*, which create evenly-spaced spectral interference notches, not global lowpass-filtering; Moss and Zageski, 1994; Simmons and Chen, 1989). We form a new sonar beam using the ratio of FM2 to FM1 in echoes compared to their relative strengths in the broadcast (Fig. 1E). While echoes arriving from a nearby zone about ±20° wide

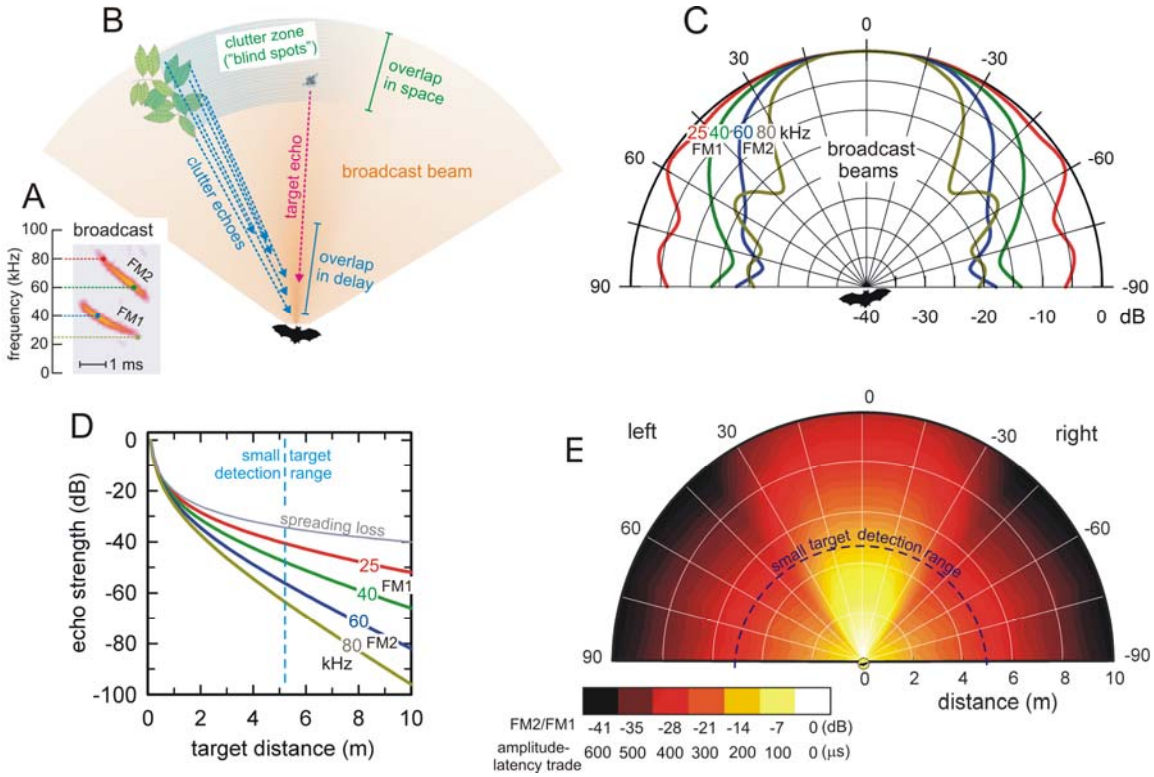


**Fig. 1.** (*A*) FM biosonar sound with harmonics FM1, FM2. (*B*) Wide broadcast beam (pink sector) ensonifies target to front as well as clutter (vegetation) to sides. Overlapping echoes form clutter zone (green shading) with "blind spots" that mask the target. (*C*) Horizontal beamwidth is narrower for FM2 (60 kHz, blue; 80 kHz, tan) than FM1 (25 kHz, red; 40 kHz, green). (*D*) Echo amplitude declines as 1/distance$^2$ due to spreading (gray), but atmospheric absorption is stronger for FM2 than FM1. (*E*) Color-density contour plot showing amplitude of FM2 relative to FM1 (0 to -41dB) according to direction and distance. Associated amplitude-latency trading retards neural responses to FM2 more than responses to FM1 (0 to 600 μs). Distance of detection for insect-sized targets is ~5 m (dashed lines in C, D).

have a “flat” spectrum (FM1 and FM2 have same relative strengths), echoes arriving from the sides or farther away have lowpass spectra (FM2 weaker than FM1). The bat’s operating range for insect-sized targets at about 5 m (Fig. 1DE; Kick, 1982). Can big brown bats exploit this harmonic beam by registering lowpass filtering in echoes to categorize clutter and mitigate masking?

## RESULTS AND DISCUSSION

We use a 2-choice experimental design based on psychophysical measurements of echo-delay discrimination (Fig. 2A; Bates and Simmons, 2011; Simmons et al., 1990; Stamper et al., 2009). (Materials and Methods are available as supporting material at *Science* online.) Probe echoes (clutter) are presented at variable delays to assess masking of test echoes (target) at a fixed delay. Masking shows as increases in discrimination errors when probe echoes fall within ±50 μs of test echoes (Simmons, 1973). Masking also depends on the horizontal separation of the test and probe echo sources (Sumer et al, 2009), but, even when the objects are separated by 40°, masking still occurs if the delays are similar (Simmons, 1973; Simmons et al., 1989). The bat’s broadcasts (Fig. 2B), are picked up by microphones (m), delayed electronically, and delivered back to the bat from two loudspeakers (s) to simulate targets at different ranges (at 58 μs/cm). The rewarded stimulus (S+) is the test echo (Fig. 2C), a 2-glint simulated target (glint delays of 3160 and 3460 μs, for a 300-μs glint separation). (Delays simulate target ranges of 55 and 60 cm.) The unrewarded stimulus (S-) is the probe echo (Fig. 2E-F), presented at variable delays (in steps from 3660 down to 3060 μs) to locate those delays where S- masks S+. (Data from 4 big brown bats; 150 trials per bat per value of S- delay.) In Fig. 3A (gray

curve for flat-spectrum S-; Fig. 2D), there are two error peaks ±50 µs wide, separated by 300 µs, that correspond to alignment of probe echoes with glint 1 and glint 2 of the test echoes (Bates and Simmons, 2010). These peaks illustrate masking, or blind spots (Fig. 1B).

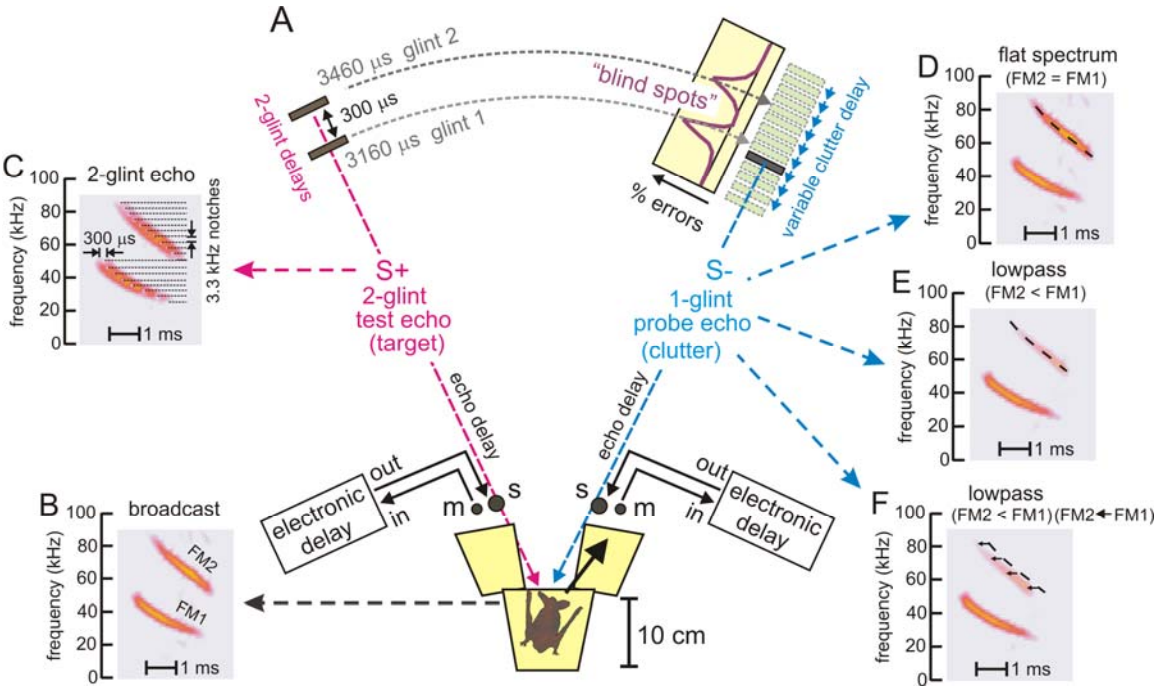


**Fig. 2.** (*A*) Two-choice behavioral experiment used to measure masking for clutter interference. Bat sits on Y-shaped platform (yellow) and emits sounds that are picked up by microphones (m), delayed electronically, and returned as simulated echoes from loudspeakers (s). Bat is rewarded for moving forward toward S+ echoes (black arrow). (*B*) Broadcast sound containing FM1, FM2. (*C*) 2-glint S+ echo with multiple interference notches at 3.3-kHz intervals due to 300-µs separation of glints. (*D*) 1-glint flat-spectrum S- echo used to demonstrate masking of S+ (inset graph in *A* shows masking effect as error peaks). (*E*) Lowpass-filtered S- with FM2 weaker than FM1 by 1.5 dB. (*F*) Lowpass-filtered S- with FM2 at earlier delays relative to FM1 to compensate for amplitude-latency trading.

First, we introduced electronic filters to separate FM1 from FM2 in S- for individual manipulation (Stamper et al., 2009) without changing FM1 and FM2 otherwise (Fig. 2D). Masking occurs around ±50 μs of either glint (Fig. 3A, green curve). Second, to determine whether harmonics are involved in clutter resistance, S- was mildly lowpass-filtered (Fig. 2E) to attenuate by 1.3 dB at 70 kHz in FM2 relative to 35 kHz in FM1, and 1.7 dB at 80 kHz in FM2 relative to 40 kHz in FM1 (mean FM2 attenuation 1.5 dB). This mimics echoes arriving from clutter located 18-20° off-axis (Fig. 1C,E). The bat's performance shows no error peaks for either glint in S+ (Fig. 3A; pink curve). Probe echoes with lowpass spectra corresponding to an off-axis location only 18-20° to the side do not mask the presence of 2-glint echoes that have spectra corresponding to a location to the front. The flat-spectrum and lowpass probe echoes deliver approximately the same echo energy to the time-window when S- has the same delay as either glint in S+, but slightly weakening FM2 in S- abolishes the masking effect (green *vs* pink curves on expanded plot in Fig. 3B).

The finding that led to our third experiment is that, when FM2 is delivered at a longer delay than FM1 by 300 μs, error peaks in performance broaden from ±50 μs to a total span of about 1000 μs (red curve in Fig. 3A; Stamper et al., 2009). The error peaks for each harmonic become nearly submerged in a broad pedestal of errors when the split-harmonic S+ is used as the test echo. In effect, the bat loses so much acuity for perceiving the delay of S+ that it blurs, or *defocuses*, its delay image. Temporal dissociation of FM2 after FM1 by as little as 3 μs is sufficient to initiate defocusing, which reaches its maximum for 5-25 μs (Fig. 3C, red curve compared with yellow curve for control) (Bates and Simmons, 2011). The defocusing effect depends specifically on

temporal misalignment of *neural responses* to FM2 in relation to FM1. Changing the amplitude of FM2 by 3 dB relative to FM1 retards FM2 responses due to *amplitude-latency trading* (Bodenhamer and Pollak, 1981). In big brown bats, the trading ratio is

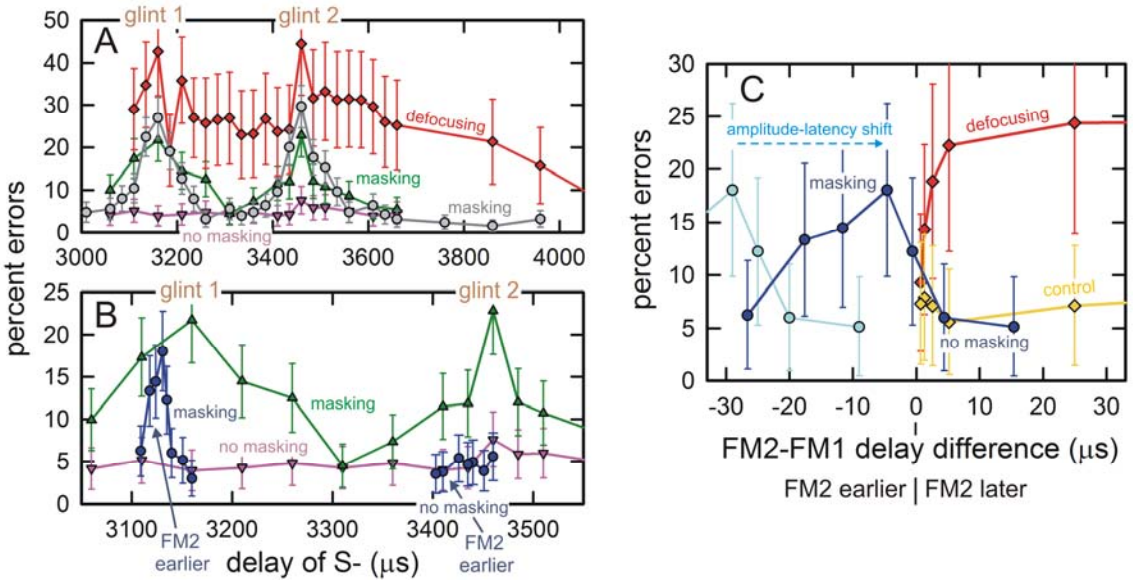


**Fig. 3.** (*A*) Normal masking (gray circles) shows as discrete error peaks when delay of flat-spectrum S- echoes (Fig. *2D*) matches the delays of 2-glint S+ echoes (Fig. *2C*). Masking persists with electronic filters that separate FM2 from FM1 in a narrow band around 55 kHz (green triangles). Lowpass filtering of S- (Fig. *2E*) abolishes masking (pink triangles). When S+ is split-harmonic echo with FM2 delayed 300 μs after FM1, masking still is present (red diamonds), but error peaks widen and are surrounded by wide plateau of errors, illustrating defocused delay image. (*B*) Expanded plot of masking by flat-spectrum S- (green triangles) and no masking by lowpass S (pink triangles) from *A*. When delay of FM2 is advanced by 0-50 μs relative to FM1 to counteract amplitude-latency trading (~24 μs) caused by 1.5-dB lowpass attenuation of FM2, masking is restored for glint 1 (dark blue circles) but not for glint 2. (*C*) Defocusing effect shown in A is activated by FM2 delays as small as 3 μs (red diamonds compared to yellow diamonds). Masking recovery data from *A* are replotted (light blue circles) and then shifted rightward by 24-μs (dark blue circles) to compensate for amplitude-latency trading. This is to align FM2 to same delay of FM1 (0 μs) as in defocusing experiment. Defocusing effect builds up for FM2-FM1 differences from 0 to 5 μs while masking declines in reciprocal fashion. All plots show mean % errors ±1 standard deviation from binomial distributions.

about 16 μs of additional latency per dB attenuation (Burkard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990). For example, attenuation of FM2 by 3 dB relative to FM1 retards its neural responses by about 48 μs relative to FM1, enough to cause defocusing (Bates and Simmons, 2010). Shifting FM2 forward in time by 48 μs compensates for amplitude-latency trading and restores focused delay acuity (Bates and Simmons, 2010).

Is defocusing of delay images associated with the absence of masking (Fig. 3A, pink curve)? In the lowpass S- echoes that mimic clutter located 18-20º off to the side (Fig. 2E), attenuation of FM2 relative to FM1 averages 1.5 dB. Amplitude-latency trading should retard neural responses to FM2 approximately 24 μs longer than responses to FM1, which is enough to cause defocusing (red curve, Fig. 3C) (Bates and Simmons, 2011). We presented the bat with S- at a delay of 3160 μs (which masks glint 1) or 3460 μs (which masks glint 2), applied lowpass filtering to remove masking, and then compensated for amplitude-latency trading by sliding FM2 to earlier delays of 0-50 μs relative to FM1 (Fig. 2F) while keeping FM1 at one or the other fixed delay. If defocusing is removable by adjusting the harmonics so that their neural responses occur in correct temporal alignment (Bates and Simmons, 2011), will masking be restored when FM2 leads FM1 by about 24 μs? Masking indeed recovers (*i.e.*, errors again occur) for glint 1 in S+ when FM2 is advanced by 25 to 45 μs (Fig. 3B, dark blue curve at glint 1). Masking does not recover for glint 2 in S+ (Fig. 3B, dark blue curve at glint 2). The 300-μs delay of glint 2 relative to glint 1 is not represented by the timing of neural responses evoked directly by glint 2 echoes but instead by the series of evenly-spaced notches in the 2-glint spectrum at 3.3-kHz intervals (Fig. 2C; Simmons et al., 1990).

Fig. 3C compares the delay differences between FM2 and FM1 that cause defocusing (red curve; Bates and Simmons, 2011) with the advancement of FM2 required to compensate for amplitude-latency trading and restore masking for glint 1 (light blue curve). First, the relative timing of neural responses to the harmonics in the masking experiment needs to be realigned to match the relative timing of responses to harmonics in the defocusing experiment, where echoes have a flat spectrum except for spectral interference notches (Fig. 2C), not lowpass filtering (Fig. 2E). To do this, the masking recovery results (Fig. 3C, light blue curve) are shifted 24-μs rightward (Fig. 3C, dark blue curve). Masking of S+ by S- occurs as a trade-off in relation to defocusing of the delay image for S+ (Fig. 3, dark blue *vs* red curves).

To prevent masking of echoes from objects located immediately to the front, big brown bats exploit the harmonic structure of their broadcast beam (Fig. 1E) to impose categorical perception on "clutter," which is defocused in perception, as distinct from "target," which is focused. Although flat-spectrum echoes can mask perception of target glints (Fig. 3A, gray curve, Fig. 3AB, green curve), clutter echoes are lowpass filtered by their location in the harmonic beam and rendered both defocused and not capable of masking (Fig. 3C). The bat's suppresses clutter masking by recruiting acoustic, neuronal, and perceptual processes into a well-integrated mechanism. Modeling of this system offers promise for technological advances in sonar design related to operation in complex surroundings.

ACKNOWLEDGEMENTS

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SUPPORTING ONLINE MATERIAL

MATERIALS AND METHODS

**Subjects**

Four adult big brown bats (Chris, Vlad, Apollo and Marina; two males and two females) were trained for this experiment. Bats were wild-caught in Rhode Island and housed individually in a temperature and humidity controlled room on a 12:12 dark:light cycle. They were given vitamin-enriched (Poly-Vi-Sol) water *ad libitum* and fed enough mealworms (*Tenebrio molitor* larvae) daily to maintain their weights between 14.5 and 15.5 g. Animal care procedures were consistent with guidelines established by the National Institute of Health and were approved by Brown University Animal Care and Use Committee.

**Procedure**

The four bats completed a two-alternative forced-choice task. The experimental chamber was a 4 m x 3 m x 2.5 m room with panels of sound-absorbent foam (Sonex, Illbrook, Inc.) lining the floor and walls. Light levels were kept dim (50 lux) during training and data collection. The equipment for producing the electronic echo stimuli was located in an adjacent room.

Each bat was trained to sit at the base of an elevated Y-platform and broadcast its echolocation sounds toward two ultrasonic microphones (Bruel & Kjaer Model 4138 "1/8-inch" condenser microphones), one located on each end of the platform. As shown in Fig. 1, microphones were mounted 20 cm away and separated by 40°. Echolocation sounds emitted by the bat were picked up by the microphones, filtered and delayed electronically and then delivered back to the bat from small electrostatic loudspeakers 15 mm in diameter (RCA, Model 112343). Speakers were mounted next to the microphones at the ends of both arms of the platform, 20 cm away from the bat and 50° apart. The bat was rewarded with a piece of mealworm for walking down the arm of the Y-platform corresponding to the loudspeaker that delivered the positive stimulus (S+), which was presented on either the left or right side in a pseudorandomized Gellerman sequence (Gellerman, 1933). If the bat made an incorrect response (choosing the negative stimulus, S-), a broadband sound was made to signal to the bat that it made an error. All trials were run using a double-blind procedure. Two experimenters were present while bats were run, a trainer who handled the bat and was blind to the position of the correct choice and a recorder who controlled which loudspeakers generated the stimuli and recorded the bat's response. The recorder observed the bat using a black and white CCD video camera (Supercircuits, Inc., Type 15-CB22-1) mounted on the ceiling above the Y-platform.

Illumination for the camera was provided by two infrared LED panels (Supercircuits, Inc.) located on either side of the video camera. The recorder was able to monitor the bat's performance on a Sony digital 8-mm video walkman recorder located behind the trainer and the Y-platform.

Sets of 50 trials were conducted each day for three days for every condition, alternating between control and experimental trials on each day (see Electronic Stimuli below). Each bat completed 150 trials over three days for each negative stimulus condition and the percentage of correct responses was recorded.

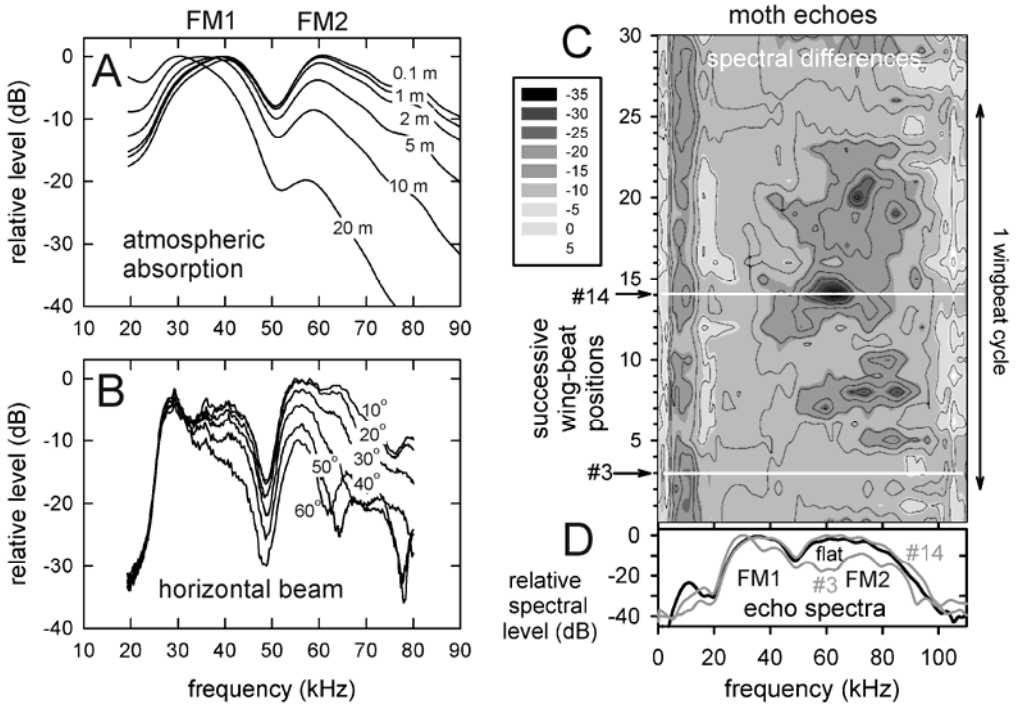
**Electronic Stimuli**

Details of the two-channel electronic target simulator system have been described fully elsewhere (Bates and Simmons, 2010; Simmons et al., 1990a, b; 2003, 2004; Stamper et al., 2004); no changes were made for the experiments described here. The total gain of this system is approximately -35 dB for S+ and -40 dB for S-. All echoes were highpass (HP) filtered at 15 kHz and lowpass (LP) filtered at 99 kHz to set the boundaries of the echo band and remove low frequency background noise.

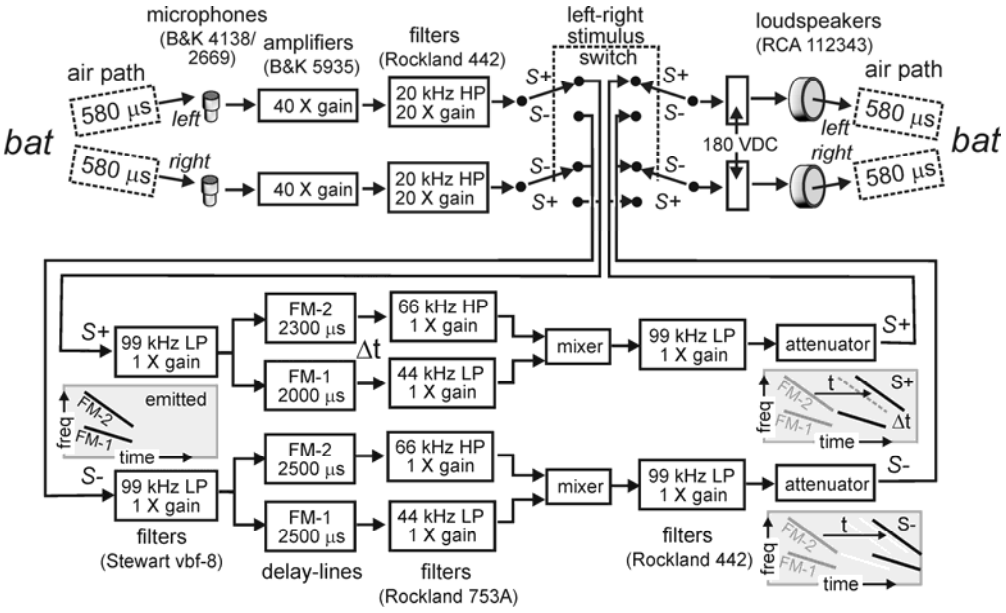
The rewarded test stimulus (S+) was a two-glint simulated target with glint delays of 3160 and 3460 μs, for a 300-μs glint separation. The unrewarded stimulus (S-) was a probe echo presented at variable delays in steps from 3660 to 3060 μs to locate the delays where S- masks perception of S+. Three types of S- were tested, corresponding to the three experiments we enumerate in the manuscript. First, flat-spectrum S- were one-glint echoes filtered in the same manner as S+ (HP 15 kHz, LP 15 kHz) with no additional manipulations to the harmonics. Second, in the lowpass-filtered S- condition, S- was

mildly lowpass-filtered to attenuate by 1.3 dB at 70 kHz in FM2 relative to 35 kHz in FM1, and 1.7 dB at 80 kHz in FM2 relative to 40 kHz in FM1 (mean FM2 attenuation 1.5 dB). The third S- was designed to offset the amplitude-latency trading caused by the attenuation of FM2 relative to FM1 in the lowpass-filtered S- condition. In this final S- condition, S- was delivered at a delay of 3160 μs (which masks glint 1) or 3460 μs (which masks glint 2). Then the echo was lowpass filtered as in our second experiment while FM2 was slid to earlier delays to compensate for amplitude-latency trading. FM2 was moved around 0-50 μs relative to FM1 while keeping FM1 at one or the other fixed delay.

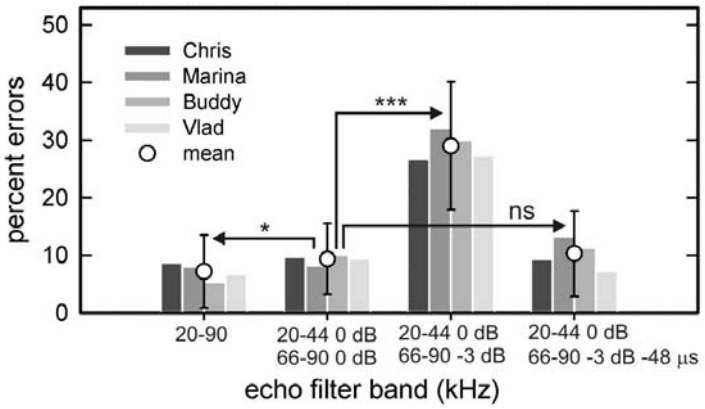
SUPPLEMENTAL FIGURES



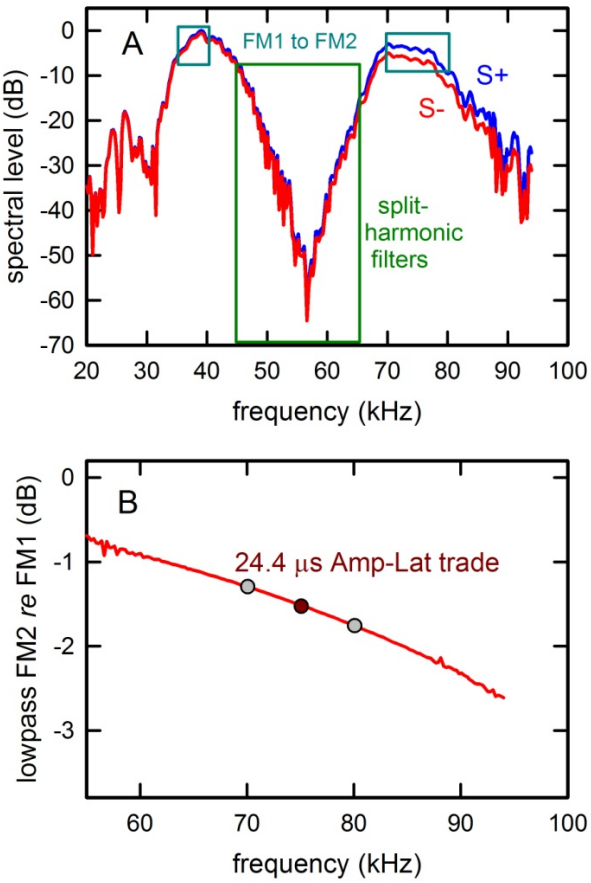

**Supplemental Fig. 1.** Spectra of FM echoes returned to bat from different distances (*A*), from different horizontal directions (*B*) and from a fluttering moth typical of the size of prey for big brown bats (*C*; moth data from Moss and Zagaeski, 1994). Echoes arriving from outside of a zone roughly 1-2 m in range and 10-20° in width have pronounced lowpass filtering (FM2 < FM1) on a scale sufficient to evoke amplitude-latency trading (16 μs latency retardation/dB attenuation; Ferragamo, et al., 1990; Burkard and Moss, 1994; Ma and Suga, 2008) and trigger the perceptual effect described in this manuscript. In contrast, echoes from fluttering moth have variable spectral shape due to shifting interference notches from overlapping glint reflections, but not overall lowpass or highpass filtering (see also Houston, et al., 2004). Insect echoes have flat spectra apart from glint interference.




**Supplemental Fig. 2.** Experimental apparatus for electronic target simulation in two-choice echo delay discrimination tests. Diagram shows electronic echo simulation system for delivering echoes as stimuli with split harmonics at $\Delta t = 300$ μs. The harmonics were segregated into different channels (FM1 and FM2), delayed by different mounts, and then mixed together to construct S+ and S-. For S+, the split harmonic bands were delayed electronically by 2300 μs for $FM_2$ and 2000 μs for $FM_1$, respectively. For S-, the split harmonic bands were delayed electronically by the same amount that varied at delay steps from 3200 μs down to 1950 μs. The example shown here gives the electronic delay of S- at 2500 μs.




**Supplemental Fig. 3.** Bar graph showing performance on delay-discrimination tasks at 800 μs index point (percent errors) for 4 bats (150 trials/bat, with mean performance ±1 SD shown by circles). Introduction of harmonic-split filters with no other change causes small, significant decrease in acuity (more errors) compared to full-band echoes (* = P< 0.05). Decreasing FM2 by 3 dB (with associated amplitude-latency trade of 48 μs) causes further, large decline in performance (*** = P< 0.001), but advancing FM2 in time by 48 μs compensates for amplitude-latency trading and restores performance (ns = P> 0.05). Results show no evidence for perceptual effect from 3 dB loss of FM2 except for the associated latency shift. (From Fig. 5B in Bates and Simmons, 2010).




**Supplemental Fig. 4.** Lowpass characteristic for amplitude difference between FM2 and FM1 in S+ (for pink circles in Fig. 4 B,C,D and Fig. 5) shows attenuation of 2nd harmonic frequencies in FM2 relative to corresponding 1st harmonic frequencies in FM1. Differential attenuation is 1.3-1.7 dB from 70 to 80 kHz. The corresponding mean expected amplitude-latency trading for responses to FM2 *re* FM1is (at a ratio of -16 μs/dB) about 24 μs.

# GENERAL DISCUSSION

Echolocating big brown bats (*Eptesicus fuscus*) emit wideband frequency-modulated (FM) biosonar sounds that span frequencies from about 22 kHz to 105 kHz in several downward-sweeping harmonics (FM1 ~55-20 kHz, FM2 ~105-45 kHz; Saillant et al., 2007; Surlykke and Moss, 2000). The sounds travel outward to impinge on objects at different distances and form echoes that return to the bat at different delays, from which the bat can judge the distance to each reflecting object (Simmons et al., 1995).

Big brown bats are highly social, and have been observed to forage near conspecifics and even engage in lengthy aerial chases with other bats (Simmons et al., 2001). In the presence of other echolocating conspecifics, they face the potential problem of acoustical interference from neighbors utilizing the same range of frequencies in their echolocation signals. Yet videos of foraging big brown bats indicate that they have little problem orienting and capturing prey in the presence of many other echolocating bats (Simmons et al., 2001). Animals that emit their own orienting signals could adapt by changing the frequencies of their signals in the presence of interference in order to avoid masking or "jamming" from conspecifics. However, each bat uses about the same frequency ranges so that changing frequency can only affect the margins of the spectrum. The problem then is whether bats do change their frequencies and, if so, how this affects their perception.

In the first paper of this dissertation, I presented evidence collected from free-flying bats in the field and pairs of bats in a laboratory flight room that supports the existence of a jamming avoidance response (JAR) in big brown bats. When flying in small groups of two to four bats in the field, variance in ending FM1 frequency increased.

In the flight room, bats that emitted sounds of similar frequency when flying singly bidirectionally shifted the frequency of their sounds when flying together so that starting and ending FM1 frequencies were more different. However, bats that began with more dissimilar sounds did not shift their frequencies when flying together. This suggests that while big brown bats can exhibit a JAR when flying with conspecifics in certain circumstances, it may not always be necessary. It also indicates that only slight differences in overall frequency content are sufficient to prevent interference in perception.

It is likely that bats possess several mechanisms for dealing with this type of potential acoustic interference. Suga et al. (1983) listed several such possibilities, based on bat neurophysiology, including: 1) the directionality of the bat's emissions, 2) the directional sensitivity of the bat's ear, 3) binaural processing, 4) sequential processing of echoes, and 5) an auditory time gate for echo processing. Behavioral studies have also revealed strategies that echolocating bats may use to reduce jamming in the presence of conspecifics. A commonly observed mechanism is to alter the duration of pulses or the interpulse interval to avoid overlapping the sounds produced by nearby bats (Obrist, 1995; Surlykke and Moss, 2000). Finally, bats that travel the same route from roost site to foraging grounds night after night (Rydell, 1990) may be relying on spatial memory and not on the echoes of their surroundings to navigate in familiar areas (Griffin, 1958; Höller, 1995).

Shifting the frequency of biosonar broadcasts appears to be one effective strategy for avoiding jamming from the sounds of other bats. It may also be used by bats in overcoming another perceptual challenge, that of clutter rejection. Although big brown

bats are usually characterized as aerial-feeding insectivores (Schnitzler et al., 2003), video and acoustic recordings made in natural conditions reveal that they also frequently fly into vegetation while hunting for prey, while in transit from roost to hunting grounds, and while chasing each other in aerial "dogfights" (Simmons, 2005; Simmons et al., 2001). Each echolocation emission ensonifies objects located not only to the bat's immediate front (targets) but also objects located to the sides and in the background (clutter). In complex surroundings, echoes from clutter may arrive within the same time window as echoes from targets, to induce masking that prevents perception of target features.

In the second paper, I discussed how big brown bats changed the frequency of their sounds – in a manner similar to a JAR – when faced with a densely cluttered flight room. When streams of echoes from successive sounds overlapped, bats raised the frequency of the first sound and lowered the frequency of the second sound. These shifts in frequency were comparable in size to the shifts observed in the JARs. Due to the harmonic structure of the sounds, the frequency shifts had a dramatic effect on the time-frequency structure of the broadcasts and associated echoes. The consequence of small frequency shifts are passed on to the higher harmonics by the integer relations of their frequencies, magnifying the effective distance between matched echoes and mismatched echoes. In effect, spectrograms act like fingerprints of the sounds: changes in frequency move the ridges to different locations.

Big brown bats apparently use the same behavioral strategy – manipulating the frequencies in the FM harmonics to facilitate matching echoes to broadcasts - to solve two different perceptual challenges, jamming avoidance from conspecifics and rejection

of background clutter echoes. In the next three papers I explored the question of how changing the harmonic structure of echoes affects perception of echo delay. What are the perceptual consequences of these small frequency shifts, and how do bats use details in the harmonic structure of their broadcasts to recognize their own echoes?

My results confirmed that big brown bats are sensitive to very small disruptions in the time-frequency structure of the FM harmonics in returning echoes, and this sensitivity was manifested as a disproportionately larger loss in echo delay acuity. The bats perceived echoes with misaligned harmonics – even by as little as 2.6 μs – as having a poorly defined delay, while echoes with aligned harmonics are perceived with much sharper delay acuity. Most importantly, these poorly defined, or defocused, range images result in actual rejection of the smeared image by assigning it to the "clutter" category and rejecting it from further processing. This means that the bat can treat all unwanted sounds – more distant or off-axis clutter echoes, the sound-echo pairs of other bats, anything that is not the target echo – as acoustic clutter by imposing upon them a process of defocusing. In this way, the target echo becomes more salient – it is very focused and its delay can be processed with high acuity. A closer examination of the acoustic properties of sound travel in air reveal how this is accomplished.

The schematic in Fig. 1 illustrates the relation between acoustic, sound spectrograms (in blue) and auditory, neural spectrograms (in red) for echoes returning from an idealized object at different locations in the bat's broadcast beam (indicated by the light gray area that widens and fades out to the right of the bat). The sound spectrogram for a broadcast containing equal-strength FM1 and FM2 is shown at the far left (blue). This emitted sound travels outward from the bat to impinge on objects in the

environment and then returns as an echo after having undergone changes in strength and spectrum due to the effects of propagation in air and the shape of the broadcast beam. Echoes are depicted to emphasize the effects only of target location at various places inside the broadcast beam. A general decline in echo strength occurs due to geometric spreading. In addition, a progressive loss of FM2 relative to FM1 occurs for locations farther away or off-axis from the broadcast beam due to lowpass filtering from atmospheric propagation and directionality (Lawrence and Simmons, 1982). As target range becomes shorter, echoes become progressively stronger and their bandwidth increases progressively, largely due to increases in the strength of FM2 (Kick and Simmons, 1984; Lawrence and Simmons, 1982). Note that propagation and directionality cause only overall attenuation and lowpass filtering of echoes, not highpass filtering (lower right corner).

The neural spectrograms (red) shown in Fig. 1 are schematic representations of the auditory system's representation for the broadcast and the various echoes arriving from targets at different locations in the broadcast beam. They trace the time of occurrence, or response latency, of individual spikes that occur across neurons tuned to different frequencies in the sweeps of FM1 and FM2. These neural spectrograms resemble the sound spectrograms, with two important distinctions. First, the decline in overall echo strength and lowpass filtering associated with distance and direction, represented in the sound spectrograms (blue) as gradual fading and eventual loss of high frequencies in FM2 and then even FM1, is converted into progressively longer response latencies, not weaker responses (Bodenhamer and Pollak, 1981; Burkard and Moss, 1994; Ma and Suga, 2008; Simmons et al., 1990a; Simmons et al., 1990b). That is,

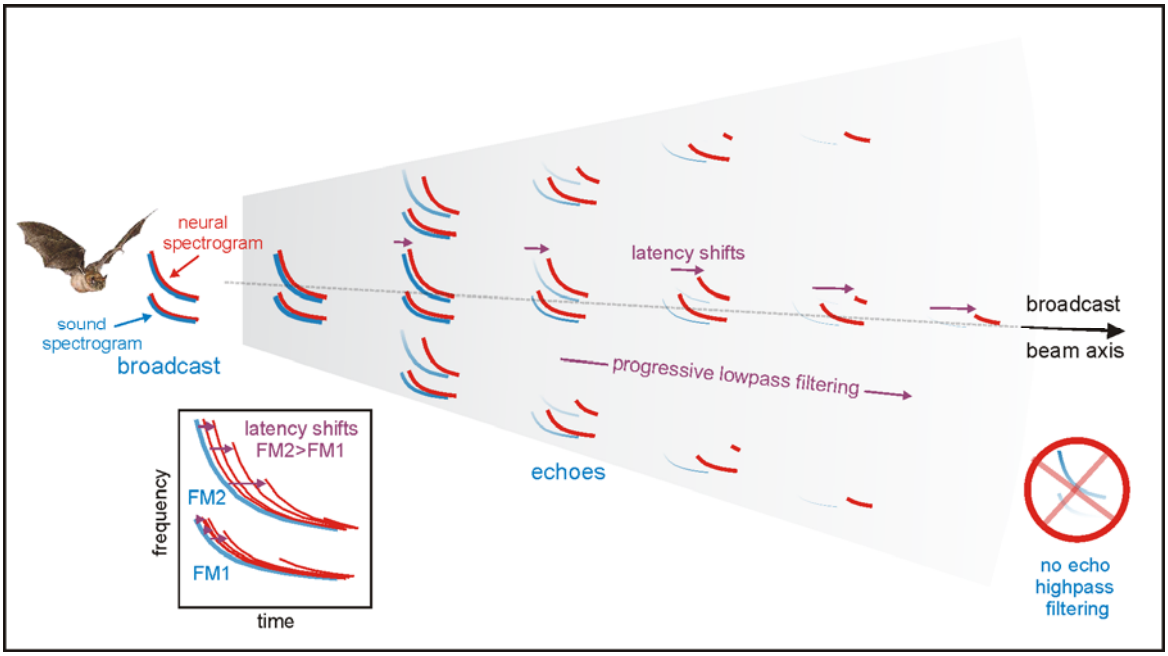



Fig. 1. Acoustic and neural spectrograms. Sound spectrogram of a big brown bat sonar broadcast (left, blue) and sound spectrograms (left to right, blue) for idealized echoes arriving from a target located at various distances and directions from the bat. The broadcast beam is indicated by a gradually widening light gray area that fades out for longer distances. The acoustic spectrograms for the echoes fade out as distance increases, or as direction departs from the axis of the broadcast beam. Frequency dependence of propagation and eccentricity in the beam both cause FM2 to fade out more rapidly than FM1. The effects of propagation and direction are entirely lowpass in nature; no highpass filtering occurs naturally (inset at lower right). The neural version of the spectrogram for the broadcast (left, red) matches the corresponding sound spectrogram (blue). It is composed of numerous single spikes in neurons tuned to successive frequencies in the FM sweeps, so that spike latencies across these responses trace the shape of FM1 and FM2. As the amplitudes of echoes decline with distance (fade out of blue curves), the corresponding neural spectrograms shift to later times (red relative to blue), a phenomenon called amplitude-latency trading. Also, as the lowpass effect of propagation or direction accumulates, more of the high-frequency end of FM2 disappears from the neural spectrograms.

the individual spikes making up the neuronal responses do not become *weaker in amplitude*, they become *later in time*. This progressive increase in response latency as stimulus amplitude decreases is known as *amplitude-latency trading*. In big brown bats, amplitude-latency trading of auditory responses for echoes is about -16 µs/dB (i.e.,

latency becomes longer by 16 μs for each 1 dB decrease in echo strength) (Ma and Suga, 2008; Simmons, et al., 1990a; Simmons et al., 1990b). The second distinction follows from amplitude-latency trading and is a consequence of the greater attenuation of higher frequencies, particularly as it is manifested as attenuation of FM2 relative to FM1. As the target's position becomes farther away or more off-axis, so that the amplitude of FM2 decreases relative to the amplitude of FM1, the neural spectrograms come to be distorted along their time axis by the progressive shift of responses to FM2 to longer latencies compared to responses to FM1 (Ma and Suga, 2008; Sanderson and Simmons, 2000; Sanderson and Simmons, 2002; Simmons et al., 1990a; Simmons et al., 1990b). For a target located at long range or off-axis, not only does the trace of FM2 occur later in the neural spectrograms than the trace of FM1, but the high-frequency end of the trace for FM2 also becomes truncated at its upper-frequency starting point. Eventually, the trace for FM2 disappears entirely. These effects are illustrated in the inset at the lower left in Fig. 1, which shows the sound (blue) and neural (red) spectrograms of echoes from different distances after they have been aligned in time and superimposed. The latency shifts to the right in the red auditory spectrograms caused by lowpass filtering are larger for FM2 than for FM1, and the upper end of the trace for FM2 gradually is lost.

Big brown bats receive echoes from objects at different distances in different directions and extract their delays to determine target range (Simmons et al., 1995). The echo spectra are involved in two ways. First, overlapping reflections from a target's glints create interference notches at certain frequencies to represent the shape of targets that are located nearby (~<1.5 m) and on the axis of the broadcast beam. The bat estimates the delay separation of the glint reflections from the frequencies of the notches

(Simmons et al., 1995). However, this process only works well for a small number of glints, which produce a well-defined sequence of notches at different frequencies. As illustrated in Fig. 1, echoes from more distant or off-axis objects undergo significant lowpass filtering. As the extent of echo lowpass filtering increases with increasing target eccentricity and distance, the bat's perception of echo delay becomes increasingly defocused by accumulation of numerous glint-delay estimates related to the wide span of frequencies removed by the lowpass effect. In effect, the bat treats the global effect of lowpass filtering as though it were caused by many local spectral notches distributed over all of the higher echo frequencies that have been attenuated. The bat considers all of them to be the locations of individual interference notches. The delay images evoked by lowpass echoes thus become defocused so that they contain many perceived delays extending over a span of several hundred microseconds, instead of the single objective delay determined by their nominal arrival time. These lowpass filtered echoes, represented by defocused images of off-axis or distant objects, do not mask the perception of echoes from nearby on-axis targets having overall flat spectra. Echoes from nearby on-axis targets evoke sharp delay images because their spectra only contain well-localized interference notches due to glints, which the bat's auditory system can efficiently process. The bat uses the defocusing effect for off-axis and distant echoes to discriminate against clutter while also perceiving nearer on-axis echoes as having well-defined delays for focused images depicting target shape.

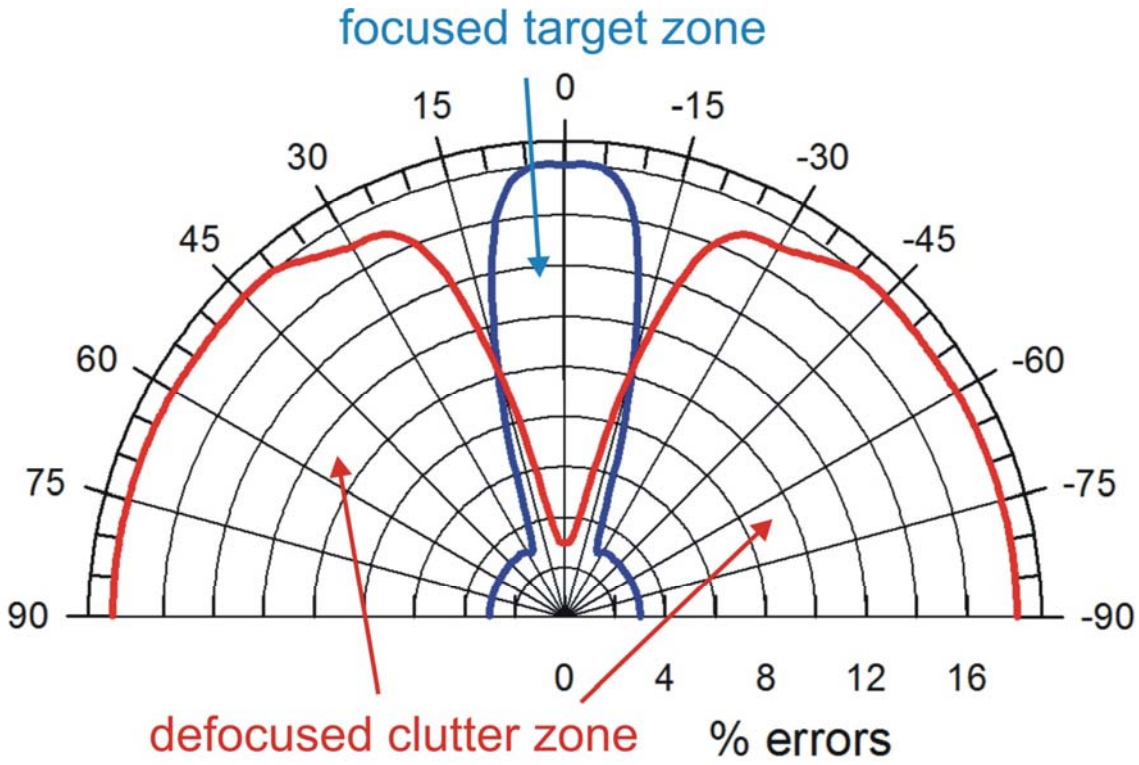



Fig. 2. Spatial representation of central focused target zone surrounded by defocused clutter zone. The blue curve shows magnitude of masking effect in terms of percent errors caused by alignment of masker with stimulus to be detected (replotted from the red curve from Fig. 5, Chapter 6). The red curve shows the defocusing effect when FM2 lags FM1 in time (replotted from light cyan curve from Fig 5, Chapter 6). Just as Fig. 5 in Chapter 6 shows a sharp transition from masking to defocusing, this polar plot shows a sharp transition in horizontal direction for the same effect plotted in angular coordinates. The translation from the horizontal scale in μs of Fig. 5 in Chapter 6 to the horizontal angle coordinates in this polar plot occurs in two steps: First, the μs scale is converted into decibels (dB) using the amplitude-latency trading ratio of 16 μs /dB. Next, the horizontal broadcast beam pattern in Fig 2B from Chapter 6 shows the relative amplitude of the beam at different frequencies and in different directions. The polar plot above was obtained by approximating the angle at which FM2 is weaker than FM1 by ~ 0.3 dB and using that point in angles as the transition point of about 3 μs from fig 5 in Chapter 6 in μs. In other words, the 3 μs FM2 to FM1 time difference at which the defocusing effect switches on is plotted in angular units in this present polar plot.

Fig. 2 is a representation of the bat's sharpness of acuity created using data from Fig. 5 in Chapter 6. It shows the central imaging zone used for high-acuity perception of targets directly in front of the bat (in blue), surrounded by a clutter zone upon which the

defocusing effect is imposed (in red). Most importantly, this plot shows a sharp boundary between the two zones, reflecting the sharp differentiation between the masking effect and the defocusing effect (red and light cyan plots in Fig. 5, Chapter 6). The spatial boundary between the central focused target imaging zone and the surrounding defocused clutter-rejection zone is created by the auditory system's capacity to detect the coherence of harmonics in broadband echoes. It exploits a unique auditory process that amplifies small differences in echo amplitude spectra to create large differences along the time axis of time-frequency representations which can then be used in jamming avoidance and clutter rejection.

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